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(54) Title: IMPROVED BACILLUS HOST CELL

(57) Abstract: A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 191, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions

Improved Bacillus Host Cell

TECHNICAL FIELD

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Bacillus sp. are attractive hosts for the production of heterologous proteins due their ability to secrete proteins directly into the culture medium. They have a high capacity for protein secretion, are genetically highly amenable, nonpathogenic and free of endotoxins, and consequently a large variety of proteins from different organisms have been efficiently produced and secreted in Bacillus sp. i.e. in Bacillus licheniformis.

In the highly competitive biotech industry, even slightly improved Bacillus host cells are in demand, which may provide more attractive production systems, or may even just be alternative production systems.

Many industrial products of commercial interest can be produced biologically in Bacillus sp. host cells e.g. heterologous polypeptides, amino acids, carbohydrates etc. Some of these products are sold as process aids, intermediates, or even end-products in the food and feed industries as well as in the pharmaceutical industry. There are increasingly strict regulations that must be complied with when producing such products in microbial production hosts for sale in these industries, for instance the presence of bacterial spores in the products is seen as a problem. When producing in Bacillus licheniformis it is thus desirable to ensure that the host cell is not capable of forming spores.

A problem to be solved by the present invention is how to obtain a Bacillus licheniformis host cell incapable of forming spores, or how to impair the sporulation process of said cell. The present invention provides a solution to the problem by providing a Bacillus licheniformis host cell which has a reduced capacity to produce one or more polypeptide(s) involved in

Accordingly, in a first aspect the invention relates to a Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129 (both included), or in SEQ ID NO's: 2 to 191 (both included), preferably at least 85% identical, more preferably at least 90% identical, still more preferably at least 95% identical, and most preferably at least 97% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129 (both included), or in SEQ ID NO's: 2 to 191 (both included), wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than

the parent host cell, when they are cultivated under comparable conditions. Preferably the mutant host cell expresses at least 10% less, more preferably at least 20% less, still more preferably at least 30% less, even more preferably at least 40% less, yet more preferably at least 50% less, or at least 60% less, or at least 70% less, or at least 80%, or most preferably at least 90% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions. Most preferably the mutant host cell expresses absolutely nothing of the one or more polypeptide(s) involved in sporulation.

Comparable conditions of cultivation must be used in order to compare the expression level of the one or more polypeptide(s) involved in sporulation in a mutant host cell of the invention with that in a parent host cell. They are cultivated separately under identical conditions in identical setups, of course allowing for the usual standard deviations of the operating parameters normally associated with growth experiments, such as temperature control etc. The quantification of the expression level of the one or more polypeptide(s) is done by standard text-book assay, techniques as known in the art e.g. mRNA quantification or immuno-based assays.

In a second aspect the invention relates to a process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in the previous aspect in a suitable medium, whereby the said product is produced.

Finally, an aspect of the invention relates to a use of a *Bacillus licheniformis* mutant host cell as defined in the first aspect for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced.

DEFINITIONS

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Nucleic acid construct: When used herein, the term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or which has been modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature. The term nucleic acid construct is synonymous with the term "expression cassette" when the nucleic acid construct contains the control sequences required for expression of a coding sequence of the present invention.

Control sequence: The term "control sequences" is defined herein to include all components, which are necessary or advantageous for the expression of a polypeptide of the present

invention. Each control sequence may be native or foreign to the nucleotide sequence encoding the polypeptide. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleotide sequence encoding a polypeptide.

Operably linked: The term "operably linked" is defined herein as a configuration in which a control sequence is appropriately placed at a position relative to the coding sequence of the DNA sequence such that the control sequence directs the expression of a polypeptide.

Coding sequence: When used herein the term "coding sequence" is intended to cover a nucleotide sequence, which directly specifies the amino acid sequence of its protein product. The boundaries of the coding sequence are generally determined by an open reading frame, which usually begins with the ATG start codon. The coding sequence typically include DNA, cDNA, and recombinant nucleotide sequences.

Expression: In the present context, the term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

Expression vector: In the present context, the term "expression vector" covers a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of the invention, and which is operably linked to additional segments that provide for its transcription.

DETAILED DISCLOSURE

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A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 191 (both included), wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions.

The term "parent host cell" in the context of the present invention means a cell which is genetically identical, or isogenic, to the progeny mutant or mutant cell of the present

invention, except for the mutated one or more gene(s) encoding one or more polypeptide(s) involved in sporulation in said mutant.

The degree of identity, or %-identity of polypeptide sequences can suitably be investigated by aligning the sequences using a computer program known in the art, such as "GAP" provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711)(Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453). Using GAP with the following settings for DNA sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3".

An object of the present invention is to provide a culture medium free of bacterial spores so as to reduce the product purification to a minimum, and to comply with regulatory requirements. This may be done according to the invention by reducing or even completely abolishing the expression of one or more gene(s) encoding a native polypeptide(s) involved in sporulation via mutagenisation of that (those) gene(s). One of the very well-known method of ensuring that a gene is not expressed into an active polypeptide within a cell is simply to delete or partially delete the encoding gene. Many techniques have been described in the art on how to specifically delete or partially delete one or more gene(s) in the genome of a cell, and certainly from the genome of a *Bacillus licheniformis* cell (see e.g. Novozymes A/S WO 01/90393, Novozymes A/S WO 02/00907). Accordingly, a preferred embodiment of the present invention relates to a host cell of the first aspect, which is mutated by a partial or complete deletion of the one or more gene(s) encoding the one or more polypeptide(s) involved in sporulation.

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A preferred embodiment of the present invention relates to a host cell of the first aspect, which is mutated in two or more genes encoding two or more polypeptides involved in sporulation.

The product of interest to be produced by the mutant host cell of the first aspect may be one or more polypeptide(s) encoded by one or more heterologous gene(s). Consequently, a preferred embodiment of the present invention relates to a host cell of the first aspect, which comprises one or more heterologous gene(s) encoding one or more heterologous polypeptide(s).

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In the industrial production of polypeptides it is of interest to achieve a product yield as high as possible. One way to increase the yield is to increase the copy number of a gene

encoding a polypeptide of interest. This can be done by placing the gene on a high copy number plasmid. However, plasmids are unstable and are often lost from the host cells if there is no selective pressure during the cultivation of the host cells. Another way to increase the copy number of the gene of interest is to integrate it into the host cell chromosome in multiple copies. Integration of two genes has been described in WO 91/09129 and WO 94/14968 (Novozymes A/S) the content of which is hereby incorporated by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) is present in at least two copies, preferably at least 4 copies, and most preferably at least 6 copies. In another embodiment the heterologous gene(s) is present in at least ten copies. If carried on a plasmid the gene(s) may be present in several hundred copies per cell, so in a still further embodiment of the present invention the heterologous gene(s) is present in at least 100 copies.

Integration of two genes closely spaced in anti-parallel tandem to achieve better stability has been described in WO 99/41358 (Novozymes A/S) the content of which is hereby incorporated by reference, as well as the stable chromosomal multi-copy integration of genes described in WO 02/00907 (Novozymes A/S) the content of which is incorporated herein by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are stably integrated into the genome of the cell.

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Selection of chromosomal integrant has for convenience resulted in the use of selectable markers such as antibiotic resistance markers. However it is desirable if possible to avoid the use of antibiotic marker genes. WO 01/90393 discloses a method for the integration of a gene in the chromosome of a host cell without leaving antibiotic resistance markers behind in the strain, the content of which is hereby incorporated by reference A preferred embodiment of the present invention relates to a host cell of the first aspect wherein the heterologous gene(s) is integrated into the genome of the cell without leaving any antibiotic resistance marker gene(s) at the site of integration.

The present invention also relates to nucleic acid constructs comprising a nucleotide sequence encoding a product of interest, which may be operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

A nucleotide sequence encoding a polypeptide ofinterest may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the nucleotide sequence prior to its insertion into a vector may be desirable or necessary depending on the expression

vector. The techniques for modifying nucleotide sequences utilizing recombinant DNA methods are well known in the art.

Other ways of increasing the product yield would be to increase promoter activity of the specific promoter regulating the expression of a specific gene of interest. Also a more general increase in the activity of several promoters at the same time could lead to an improved product yield. The control sequence may be an appropriate promoter sequence, a nucleotide sequence which is recognized by a host cell for expression of the nucleotide sequence. The promoter sequence contains transcriptional control sequences, which mediate the expression of the polypeptide. The promoter may be any nucleotide sequence which shows transcriptional activity in the host cell of choice including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

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Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention, especially in a bacterial host cell, are the promoters obtained from the E. coli lac operon, Streptomyces coelicolor agarase gene (dagA), Bacillus subtilis levansucrase gene (sacB), Bacillus licheniformis alpha-amylase gene (amyL), Bacillus stearothermophilus maltogenic amylase gene (amyM), Bacillus amyloliquefaciens alpha-amylase gene (amyQ), Bacillus licheniformis penicillinase gene (penP), Bacillus subtilis xylA and xylB genes, and prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, Proceedings of the National Academy of Sciences USA 75: 3727-3731), as well as the tac promoter (DeBoer et al., 1983, Proceedings of the National Academy of Sciences USA 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242: 74-94; and in Sambrook et al., 1989, supra.

Other useful promoters are described in WO 93/10249, WO 98/07846, and WO 99/43835 (Novozymes A/S) the contents of which are incorporated fully herein by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are transcribed from a heterologous promoter or from an artificial promoter.

The control sequence may also be a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleotide sequence encoding the polypeptide. Any terminator which is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a suitable leader sequence, a nontranslated region of an mRNA which is important for translation by the host cell. The leader sequence is operably linked to the 5' terminus of the nucleotide sequence encoding the polypeptide. Any leader sequence that is functional in the host cell of choice may be used in the present invention.

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The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3' terminus of the nucleotide sequence and which, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a signal peptide coding region that codes for an amino acid sequence linked to the amino terminus of a polypeptide and directs the encoded polypeptide into the cell's secretory pathway. The 5' end of the coding sequence of the nucleotide sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted polypeptide. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region which is foreign to the coding sequence. The foreign signal peptide coding region may be required where the coding sequence does not naturally contain a signal peptide coding region. Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to enhance secretion of the polypeptide. However, any signal peptide coding region which directs the expressed polypeptide into the secretory pathway of a host cell of choice may be used in the present invention.

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Effective signal peptide coding regions for bacterial host cells are the signal peptide coding regions obtained from the genes for Bacillus NCIB 11837 maltogenic amylase, Bacillus stearothermophilus alpha-amylase, Bacillus licheniformis subtilisin, Bacillus licheniformis beta-lactamase, Bacillus stearothermophilus neutral proteases (nprT, nprS, nprM), and Bacillus subtilis prsA. Further signal peptides are described by Simonen and Palva, 1993, Microbiological Reviews 57: 109-137.

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The control sequence may also be a propeptide coding region that codes for an amino acid sequence positioned at the amino terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to a mature active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding

region may be obtained from the genes for Bacillus subtilis alkaline protease (aprE), Bacillus subtilis neutral protease (nprT), Saccharomyces cerevisiae alpha-factor, Rhizomucor miehei aspartic proteinase, and Myceliophthora thermophila laccase (WO 95/33836).

- Where both signal peptide and propeptide regions are present at the amino terminus of a polypeptide, the propeptide region is positioned next to the amino terminus of a polypeptide and the signal peptide region is positioned next to the amino terminus of the propeptide region.
- 10 It may also be desirable to add regulatory sequences which allow the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those which cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems include the lac, tac, and trp operator systems. In yeast, the ADH2 system or GAL1 system may be used. In eukaryotic systems, these include the dihydrofolate reductase gene which is amplified in the presence of methotrexate, and the metallothionein genes which are amplified with heavy metals. In these cases, the nucleotide sequence encoding the polypeptide would be operably linked with the regulatory sequence.
- The present invention also relates to recombinant expression vectors comprising the nucleic acid construct of the invention. The various nucleotide and control sequences described above may be joined together to produce a recombinant expression vector which may include one or more convenient restriction sites to allow for insertion or substitution of the nucleotide sequence encoding the polypeptide at such sites. Alternatively, the nucleotide sequence of the present invention may be expressed by inserting the nucleotide sequence or a nucleic acid construct comprising the sequence into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.
- The recombinant expression vector may be any vector (e.g., a plasmid or virus) which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of the nucleotide sequence. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids.

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The vector may be an autonomously replicating vector, i.e., a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication,

e.g., a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome.

The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, or a transposon may be used.

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The vectors of the present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

Examples of bacterial selectable markers are the dal genes from Bacillus subtilis or Bacillus licheniformis, or markers which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance.

The vectors of the present invention preferably contain an element(s) that permits stable integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

For integration into the host cell genome, the vector may rely on the nucleotide sequence encoding the polypeptide or any other element of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleotide sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleotide sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the To increase the likelihood of integration at a precise location, the chromosome(s). integrational elements should preferably contain a sufficient number of nucleotides, such as 100 to 1,500 base pairs, preferably 400 to 1,500 base pairs, and most preferably 800 to 1,500 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding nucleotide sequences. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in E. coli, and pUB110, pE194, pTA1060, and pAMß1 permitting replication in Bacillus. The origin of replication may be one having a mutation which makes its functioning temperature-sensitive in the host cell (see, e.g., Ehrlich, 1978, Proceedings of the National Academy of Sciences USA 75: 1433).

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More than one copy of a nucleotide sequence of the present invention may be inserted into the host cell to increase production of the gene product. An increase in the copy number of the nucleotide sequence can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the nucleotide sequence where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the nucleotide sequence, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art (see, e.g., Sambrook et al., 1989, supra).

The introduction of a vector into a bacterial host cell may, for instance, be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, Molecular General Genetics 168: 111-115), using competent cells (see, e.g., Young and Spizizin, 1961, Journal of Bacteriology 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, Journal of Molecular Biology 56: 209-221), electroporation (see, e.g., Shigekawa and Dower, 1988, Biotechniques 6: 742-751), or conjugation (see, e.g., Koehler and Thorne, 1987, Journal of Bacteriology 169: 5771-5278).

A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are comprised in an operon, preferably a polycistronic operon. The term "operon" in the context of the present invention means a polynucleotide comprising several genes that are clustered and perhaps even transcribed together into a polycistronic mRNA, e.g. genes coding for the enzymes of a metabolic pathway. The transcription of an operon may be initiated at a promoter region and controlled by a neighboring regulatory gene, which encodes a regulatory protein, which in turn binds to the operator sequence in the operon to respectively inhibit or enhance the transcription. The

gene or the operon can be carried on a suitable plasmid that can be stably maintained, e.g. capable of stable autonomous replication in the host cell (the choice of plasmid will typically depend on the compatibility of the plasmid with the host cell into which the plasmid is to be introduced) or it can be carried on the chromosome of the host. The said gene may be endogenous to the host cell in which case the product of interest is a protein naturally produced by the host cell and in most cases the gene will be in it normal position on the chromosome. If the gene encoding the product of interest is an exogenous gene, the gene could either be carried on a suitable plasmid or it could be integrated on the host chromosome. In one embodiment of the invention the eubacterium is a recombinant eubacterium. Also the product of interest may in another embodiment be a recombinant protein.

The product of interest is any gene product or product of a metabolic pathway which is industrially useful and which can be produced in a bacterial cell such as a *B. licheniformis*.

In one preferred embodiment, the heterologous polypeptide(s) is an antimicrobial peptide, or a fusion peptide comprising a peptide part which in its native form has antimicrobial activity.

In another preferred embodiment, the heterologous polypeptide(s) has biosynthetic activity and produces a compound or an intermediate of interest.

Yet another embodiment relates to a host cell of the first aspect, wherein the compound or intermediate of interest comprises vitamins, amino acids, antibiotics, carbohydrates, or surfactants, and preferably the carbohydrates comprise hyaluronic acid.

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In one embodiment the heterologous polypeptide(s) is an enzyme, particularly the enzyme is an enzyme of a class selected from the group of enzyme classes consisting of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and ligases (EC 6). Preferably the enzyme is an enzyme with an activity selected from the group consisting of aminopeptidase, amylase, amyloglucosidase, mannanase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, transferase, ribonuclease, polyphenoloxidase. protease, phenoloxidase, phytase, transglutaminase, or xylanase. Preferably the enzyme is an amylase or a mannanase.

A second aspect of the invention relates to a process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in the first aspect of the invention in a suitable medium, whereby the said product is produced. One embodiment relates to a process of the second aspect, further comprising isolating or purifying the product of interest. Suitable media for the cultivation is described below as well as methods for the purification or isolation of the produced product which is an optional additional step to the process of the present invention.

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In the production methods of the present invention, the cells are cultivated in a nutrient medium suitable for production of the polypeptide using methods known in the art. For example, the cell may be cultivated by shake flask cultivation, small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (e.g., in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The media are prepared using procedures known in the art (see, e.g., references for bacteria and yeast; Bennett, J.W. and LaSure, L., editors, *More Gene Manipulations in Fungi*, Academic Press, CA, 1991).

The polypeptides may be detected using methods known in the art that are specific for the polypeptides. These detection methods may include use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, an enzyme assay may be used to determine the activity of the polypeptide as described herein.

35 The resulting polypeptide may be recovered by methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures

including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation.

The polypeptides of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing), differential solubility (e.g., ammonium sulfate precipitation), SDS-PAGE, or extraction (see, e.g., *Protein Purification*, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

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A third aspect of the present invention relates to the use of a *Bacillus licheniformis* mutant host cell as defined in the first aspect for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced, and optionally isolating or purifying the produced product.

WO 03/087148

CLAIMS

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- 1. A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 191, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions.
- 2. The host cell according to claim 1, which is mutated by a partial or complete deletion of the one or more gene(s) encoding the one or more polypeptide(s) involved in sporulation.
 - 3. The host cell according to any of claims 1 2, which is mutated in two or more genes encoding two or more polypeptides involved in sporulation.
- 4. The host cell according to any of claims 1 3, which comprises one or more heterologous gene(s) encoding one or more heterologous polypeptide(s).
 - 5. The host cell according to claim 4, wherein the heterologous gene(s) is present in at least two copies.
 - 6. The host cell according to claim 4 or 5, wherein the heterologous gene(s) are stably integrated into the genome of the cell.
- 7. The host cell according to any of claims 4 6, wherein the heterologous gene(s) is integrated into the genome of the cell without leaving any antibiotic resistance marker genes at the site of integration.
 - 8. The host cell according to any of claims 4 7, wherein the heterologous gene(s) are transcribed from a heterologous promoter or from an artificial promoter.
 - 9. The host cell according to any of claims 4 8, wherein the heterologous gene(s) are comprised in an operon, preferably a polycistronic operon.
- 10. The host cell according to any of claims 4 9, wherein the heterologous polypeptide(s) is
 35 an antimicrobial peptide, or a fusion peptide comprising a peptide part which in its native form has antimicrobial activity.

11. The host cell according to any of claims 4 - 9, wherein the heterologous polypeptide(s) has biosynthetic activity and produces a compound or an intermediate of interest.

12. The host cell according to claim 11, wherein the compound or intermediate of interest comprises vitamins, amino acids, antibiotics, carbohydrates, or surfactants.

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- 13. The host cell according to claim 12, wherein the carbohydrates comprise hyaluronic acid.
- 14. The host cell according to any of claims 4 9, wherein the heterologous polypeptide(s) is an enzyme, preferably a secreted enzyme.
 - 15. The host cell according to claim 14, wherein the enzyme is an enzyme of a class selected from the group of enzyme classes consisting of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and ligases (EC 6).
 - 16. The host cell according to claim 15, wherein the enzyme is an enzyme with an activity selected from the group of enzyme activities consisting of aminopeptidase, amylase, amyloglucosidase, mannanase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, phytase, phenoloxidase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase, and xylanase.
 - 17. The host cell according to claim 16, wherein the enzyme is an amylase or a mannanase.
 - 18. A process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in any of the claims 1 17 in a suitable medium, whereby the said product is produced.
 - 19. The process according to claim 18, further comprising isolating or purifying the product of interest.
 - 20. A use of a Bacillus licheniformis mutant host cell as defined in any of the claims 1 17 for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced.

21. The use according to claim 20 further comprising isolating or purifying the product of interest.

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Val Ser Tyr Val Asp Val Ile Lys Leu Arg Tyr Arg Asp Lys Asn Tyr 85 90 95

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Page 11

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Bacillus licheniformis

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agtcaaatat ccctataaaa aggagctgaa atccatgagc tgcggaaaac accatggccg	1188												
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Thr Ile Ile Ile Lys Asp Ser Cys Asp Val Gln Val Ser Thr Glu Asp 65 70 75

Thr Gln Thr Leu Ala Ser Val Met Thr Ala Ala Gln Thr Leu Ala Val 85 90 95

Thr Ile Ile Leu Ala Ile Ile Asp Asp Pro Asp Leu Ala Glu Leu Val 100 105

Thr Thr Asp Leu Leu Gln Val Thr Ala Asn Lys Gln Thr Asn Arg Gln 115 125

Lys Leu Val Ile Asp Asn Ser Arg Asn Val His Val Thr Thr Glu Asp 130 140

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10295.204.ST25.txt Met Met Gln Ile Val Met Gln Gly Leu Asp Asn 1 5 10

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aatgc	aaaca	. tgc	caaa	tga	caac	atcg	ac c	gcgc	cato	a aa	aaag	ctto	cgg	cagccag	1373
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Ser Arg Gly Asp Met Asn Tyr His Asn His Leu Val Asn Thr Ala Asp 50 50

Thr Gly Tyr Asp Arg Pro Glu Asn Arg Lys Ile Ser Arg Asn Ile Thr 65 70 75

Gly Arg Val Asn Lys Leu Asn Tyr Val Asp Glu Ser Gln Ala Val Val 90 95

Thr Asn Glu Thr Val Ile Ile Ala Val Arg Ser Asp Lys Arg Leu Thr 100 100

Asn Asn Glu Arg Thr Gln Ile Arg Lys Ala Ala Lys Thr Phe Ala Gly 115 120

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DNA

Bacillus licheniformis

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gtaaaaaagg agtatgaatc atg gac aca aca ttg ggc tac ctc cgt gag tca Met Asp Thr Thr Leu Gly Tyr Leu Arg Glu Ser 1	533
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atc gtt tcc ggg cga tat gcc aat gag gaa gag ttt gtc gag cac ttg Ile Val Ser Gly Arg Tyr Ala Asn Glu Glu Glu Phe Val Glu His Leu 30 35	629
gag gag cgg gaa atg gaa ttt ctg aat caa gtg ctt gaa cat gag atg Glu Glu Arg Glu Met Glu Phe Leu Asn Gln Val Leu Glu His Glu Met 45 50 55	677
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gat gaa gca gtg caa tcc ggg ctt gaa cag ctg ggc ctt cat gca gat gat gaa gca gtg caa tcc ggg ctt gaa cag ctg ggc ctt cat gca gat Asp Glu Ala Val Gln Ser Gly Leu Gln Leu Gly Leu His Ala Asp Asp Glu Ala Val Gln Ser Gly Leu Gln 20	58 1
gat gtc gaa gtc gac gta gtt gat gaa gga aaa aag gga tta ttc ggc gat gtc gaa gtc gac gta gtt gat gaa gga aaa aag gga tta ttc ggc Asp yal Glu Val Asp Val Asp Glu Gly Lys Lys Gly Leu Phe Gly Asp yal Glu Val Asp Val 35	629
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caa act tta aat gcc ctt gaa acg ctg acg cag ctc gtg ctc aat cgt Gln Thr Leu Asn Ala Leu Glu Thr Leu Thr Gln Leu Val Leu Asn Arg 115	869
cat tcc gac aga tat atc caa gcg gtg gtt gac gcc gaa gga tac cgc Page 24	917

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<213> Bacillus licheniformis

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Ser Ala Val Val Asn Ile Arg Glu Lys Ile Asp Pro Val Lys Glu Ala 50 60

Lys Gln Tyr Leu Glu Asn Val Ile Ser Asn Met Gly Ile Gln Ala Gln 65 70 75 80

Val Thr Ala Glu Glu Glu Ser Lys Arg Val Val Phe Gln Leu Lys Gly Page 25

95

85

10295.204.ST25.txt

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Thr Leu Ala Gln Leu Ala Leu Lys Leu Ala Asp Gln Ala Ala Arg Gln 145 150 160

Lys Lys Asp Ile His Leu Glu Pro Met Pro Ser Ser Glu Arg Lys Val 165 170 175

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DNA Bacillus licheniformis

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11 0 00/00/140																
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gco Ala	aca Thr 205	•	ıaaaa		aato	tga	caa a	ıcaga	ittgg	g tt	atti	gtgo	: agg	jaaat	att	1165
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²⁴ 205 PRT Bacillus licheniformis

10295.204.ST25.txt

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Tyr Ile Lys Pro Lys Lys Phe Pro Lys Leu Thr Arg Arg Cys Lys Asn 50

Phe Leu Ser Ile Thr Gln Glu Gln Val Asp Gln Gly Ile Thr Phe Asn 65 70 75

Ala Leu Ile Glu Lys Leu Arg Glu Leu Asp Pro Asp Arg Asn Ser Val 85 90 95

Ile Ile Thr Trp Gly Asn Met Asp Met Lys Val Leu Lys Gln Asn Cys 100 105 110

Met Phe Asn His Val Pro Phe Pro Phe Lys Gly Glu Met Arg Asp Leu 115 120 125

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Bacillus licheniformis

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Tyr Lys Glu Leu Phe Leu Ser Phe Glu Val Leu Glu Ile Leu Ser Val . 45 45

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Met Gly Phe Val Ile Phe Leu Thr Ile His Arg Phe Ala Leu Glu Ile 80

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Page 31

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Bacillus licheniformis

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Val Thr Ala Lys Ser Asp Arg Met Gly Tyr Arg Leu Gln Gly Glu Ala 225 230 235 240 Page 33

10295.204.ST25.txt

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Page 34

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Bacillus licheniformis

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Glu Ala Val Ile Ala Lys Gly Gly Arg Gly Arg Gly Asn Thr Arg 115 120

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Pro Gly Lys Glu Arg Tyr Ile Val Leu Glu Leu Lys Val Leu Ala Asp 145 150 160

Val Gly Leu Val Gly Phe Pro Ser Val Gly Lys Ser Thr Leu Leu Ser 165 170 175

Val Val Ser Ser Ala Lys Pro Lys Ile Ala Asp Tyr His Phe Thr Thr 180 185 190

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agi Sei	t gcg r Ala 28	a Ar	g gt: g Va	a tt 1 Ph	t gaa e Gl	a gat u Ası 290	<i>J</i> 611	a tta u Le	a tt u Ph	c ct e Le	g tg u Cy 29		g tt u Le	g ga u As	t gti p Va	1397 I
tta Lei 30	u Glu	g gc u Al	a ct a Le	g tt u Ph	t ata e Il 30	e Ly:	a tca s se	a gc r Al	t aa a As	t aa n Ly 31	3 30	a ga r Gl	g gt u Va	a ct	a gaa u Gli 31	

WO 03/087148

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Leu Ser Tyr Tyr Gln Leu Met Asn Phe Arg His Glu Leu Met Leu Glu 50 50

Tyr Leu Phe Pro Ala Glu Lys Lys Leu Ser Lys Ser Asp Tyr Leu Arg 65 70 80

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Phe Tyr His Met Lys Gln Thr His Met Ser Met Tyr Tyr Val Ser Leu 145 150 160

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Asn Cys Leu Phe Val Val Ala Gly Asn Tyr Ile Asp Leu Ser Thr His 180 185 190

Asp Gln Ala Leu Pro His Leu Leu Ser Ala Lys Glu Ser Ala Glu Asp 195 200 205

Ile Gln Asn Lys Ala Ile Val Ala Lys Ala Leu Leu Asn Val Ala Tyr 210 215 220

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Arg Asp Ser Arg Gly Tyr Pro Tyr Leu Glu Glu Leu Ala Leu Glu Ala 325 330 335

Ala Leu Phe Tyr Thr Arg Asn Glu Arg Pro Asn Asp Ser Ile Phe Phe 340 345

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ga; Gli	a aat u Asr	aaq ı Lys	g tta s Leu 33!	ועו ג	gaa Glu	ı gaa ı Glu	gcc Ala	ata 11e 340	יעי	tto Phe	tac Tyr	att lle	aaa Lys 345		gac Asp	1541
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ga	ıc								Pä	age 4	13					2077

34 358

Bacillus licheniformis

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Ser Ile Glu Met Lys Asp Asp Ile Asp Lys Ala Ile Glu Lys Met Glu 25

Glu Asp His Asp Val Leu Leu Tyr Tyr Gln Met Leu Asp Phe Arg Leu 45

Arg Leu Leu Glu Asp Ile Ser Gln Ser Ser Thr Glu Lys Leu Glu 50

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Asn His Thr Glu Ala Leu Asn Phe Phe Arg Ile Ala Glu Lys Arg Leu 105

Ser Val Ile Gln Asn Glu Ile Glu Lys Ala Glu Phe His Tyr Lys Ile 115

Gly Val Leu Tyr Tyr Asn Leu Lys Ala Thr Trp Leu Ser Ile His His 130 135

Ile Asn Ile Ala Ser Gly Ile Phe Gln Gly Tyr Asp Gly Tyr Ala Lys 145 150

Arg Val Ile Asn Cys Lys Met Leu Ile Gly Leu Asn Tyr Ile Asp Gln 175

Phe Lys Phe Ala Glu Ser Glu Val Leu Leu Lys Glu Ala Ile Glu Lys 180 185

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581

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Lys Ala Gln Glu Val Asn Ser Glu Ile Phe Glu Leu Lys Phe Lys Thr 275 280 285

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WO 03/08/140			
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	gga gct tat ga Gly Ala Tyr Gl 10	a gtg tat aaa aag aat tat gag u Val Tyr Lys Lys Asn Tyr Glu O 105	821
	tat aaa att gc Tyr Lys Ile Al 115	c gag aag aag ctt gct cat gta a Glu Lys Lys Leu Ala His Val 120	869
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•		Page 46	

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100

Tyr Lys Ile Ala Glu Lys Lys Leu Ala His Val His Asp Glu Ile Glu 125

Val Ala Gln Phe His Asp Lys Val Gly Lys Leu Tyr Tyr Tyr Leu Gly 130

Gln Asn Ile Val Ser Leu Asn His Thr Arg Gln Ala Met Glu Ile Phe 150 150

Lys Gly His Gly Asp His Asp Met Asn Leu Val Ser Thr Tyr Ile Thr 175

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Tyr Leu Thr Glu Ala Ile His Thr Val Arg Lys Ala Gly Asp Cys Phe 205

Lys Glu Met Gln Leu Leu His Asn Phe Ala Leu Leu Tyr Ala Ala Met 210 210

Asp Asn Ser Glu Lys Ser Ile Gln Phe Leu Glu Ile Val Leu Asp Asp 235 230

Gln Ala Tyr Ala Ala Ser Asp Tyr Tyr Phe Asn Ala Val Phe Leu Met 255

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His His Gly Tyr His His Gln Pro Met Thr Ala Pro Ala Tyr Ala Pro

Page 51

10295.204.ST25.txt 270 260

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Pro Gly Glu Gly His Leu Gln Lys Tyr Arg Asn Ala Lys Ser Thr Leu 50 60

Val Ile Gly Val Arg Lys Thr Leu Lys Phe Asp Ser Ser Lys Pro Ser 80

Ala Glu Tyr Ala Ile Pro Phe Ala Thr Gly Cys Met Gly His Cys His 90 95

Tyr Cys Tyr Leu Gln Thr Thr Met Gly Ser Lys Pro Tyr Ile Arg Thr 100 100 110

Tyr Val Asn Val Glu Glu Ile Leu Glu Gln Ala Asp Gln Tyr Ile Lys 115 120

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Page 55

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80

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PCT/DK03/00200 WO 03/087148

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Ser Leu Leu Lys His Leu Asp Met Thr Gln Glu Gln Leu Ala Lys A 130 · 135	
Leu Gly Lys Ser Arg Pro His Ile Ala Asn His Leu Arg Leu Leu T 155 145 page 62	hr 60

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Page 64	•							

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Cys Ser Lys Met Thr Ser Leu Ile Ile Lys Asp Arg Val Arg Thr Leu 115 120

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Gly Ser Leu Leu Ile Ser Leu Tyr Gly Glu Gly Ser Ala Glu Leu Val 290 295

Pro Thr Leu Tyr Glu Ser Leu Ile Ala Ile Gly Leu ⊅he Leu Leu Thr 305 310

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1511 <211>

DNA Bacillus licheniformis

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Glu Asp Leu Phe Thr Thr Tyr Arg Met Glu Met Asp Asp Gln Arg Ser 100 105 110

Arg Glu Arg Glu Glu Leu Thr Glu Ile Val Arg Ser Asp Lys Ala Thr 115 120 125

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Tyr

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cag gcc ttt tta gca gat ctt aaa caa ttg aaa aac agc tcg ccg aaa Gln Ala Phe Leu Ala Asp Leu Lys Gln Leu Lys Asn Ser Ser Pro Lys 205 210	1157
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tca gaa ccg att att tca agc ttt tcc gac cgt gat gaa aag ccc gaa Ser Glu Pro Ile Ile Ser Ser Phe Ser Asp Arg Asp Glu Lys Pro Glu 265 Page 90	1301

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gtt Val	caa Gln	gct Ala 270	tac Tyr	gaa Glu	gct Ala	Pro	909 Ala 275	Ala	Pro	Ala	Glu	Pro 280	Pro	Ăla	ดีโน้		
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gac Asp	cgg Arg	ccg Pro 430) Asp	gca o Ala	a aag a Lys	g ct <u>c</u> s Lei	atg Met 435	TIE	ggc Gly	cto Leu	ggo Gly	cgg / Arg 440	, ,,,,,,	att ıIle	tcc Ser		1829
gga Gly	gaa Glu 44!	ı Ali	g gta a Va	a ttg] Lei	g gca u Ala	a gag a Gli 450	ı rec	aac I Asr	aaa Lys	a atg s Met	g ccc Pro 45!		c cti	t cti u Lei	gtt val	•	1877
gca A1a 460	a GI	a gc	g ac a Th	c gg r Gl	a age y se 46	[GI	g aaa y Lys	a ago s Ser	gto Va	tg1 Cys 470	. · · · ·	c aa l Ası	c ggg n Gly	g ato y Ilo	att e Ile 475		1925
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at Il	t ga e As	t cc p Pr	g aa o Ly 49	5 ме	g gt t Va	c ga 1 Gl	g cto u Leo	c aa u Ası 50	ıı va	c ta 1 Ty	c aa r As	c gg n Gl	g at y Il 50	= ' '	g cat o His		2021
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aa Lÿ	g aa s Ly 52	s va	c gt 1 va	c aa 1 As	ic ga in Gl	a at u Me 53	ב טו	g cg u Ar	y Ai	y iy	53	=	g tt u Ph	t to le Se	t cac r His		2117
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gtc Val	ga1 Asi 68	רו כ	c gt s Va	c ate	c ago e Sei	cag Glr 690	1 911	aaa Lys	gco Ala	caa Glr	tad Tyi 695	_	ı gaa ı Glu	ı gaa ı Gli	a atg [.] u Met	2597
att Ile 700	: Pr	a ga o Gl	a ga u Gl	g ac u Th	g cag r Gli 70	1 611	a acg u Thr	g gtg Va	ago Sei	gaa r Glu 710		g aca 1 Thi	a gad r Asp	gae As	c ctt c Leu 715	2645
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Leu Gly Leu Phe Leu Thr Gly Leu Ser Leu Phe Trp Lys Lys Lys Thr 65 70 75 80

Pro Ser Phe Leu Thr Arg Arg Lys Ala Gly Leu Tyr Cys Ile Ile Ala 85 90 95

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Leu Leu Ile Thr Asp Arg Ser Leu Gln Glu Thr Leu Ile Lys Trp Met 180 185

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Lys Leu Met Ile Gly Leu Gly Arg Asn Ile Ser Gly Glu Ala Val Leu 445 435

Ala Glu Leu Asn Lys Met Pro His Leu Leu Val Ala Gly Ala Thr Gly 450 455

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Met Lys Tyr Leu Gly Leu Met Trp Ile Leu Gly Ile Ser Ile Ile Gly 85 90 95	
Leu Pro Val Ile Phe Ile Met Val Phe Leu Lys Gly Ile Val Val Gly 100 105 110	
Phe Thr Val Gly Phe Leu Val Asn Gln Met Gly Ile Asn Gly Phe Phe 115 120 125	
Leu Ser Phe Val Ser Val Leu Pro Gln Asn Ile Leu Leu Ile Pro Ala 130 135 140	
Tyr Leu Ile Met Gly Thr Cys Ala Ile Ala Phe Ser Met Arg Leu Ile 145 150 160	

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10295.204.ST25.txt

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259

307

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403

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499

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165

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gga aaa aca ttt gat gtt tcc gca tct cta agc ggt acg gtc atc aaa 547

Gly Lys Thr Phe Asp Val Ser Ala Ser Leu Ser Gly Thr Val Ile Lys

145

140

547

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10295.204.ST25.txt
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Val Ile Lys Ala Ala Lys Asp Pro Val Leu Gly Tyr Val Val Glu Val 155 160

Glu His Glu Asp Gly Leu Ser Thr Val Tyr Gln Ser Leu Ser Glu Val 175

Ser Val Lys Gln Gly Asp Lys Ile Glu Gln Asn Gln Val Ile Gly Lys 180

Ala Gly Lys Asn Leu Tyr Asn Glu Glu Gly Gly Asn His Val His Phe 195 200

Glu Ile Arg Lys Asp Gly Val Ala Leu Asn Pro Leu Asn Phe Met Asp 210

Lys Pro Val Ser Ser Ile Glu Lys Ala Met Glu Glu Gln Ala Ser Glu 230 235

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ctt tat Leu Tyr 45	gtc atc Val Ile	ggc gcc Gly Ala	gtg ata Val Ile 50	tat Tyr	tgg acg Trp Thr	cac ga His As 55	at ccg sp Pro	cag Gln	tca Ser	677
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and analysis of the second sec	1674
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Leu His Tyr Leu Ile Val Ala Val Phe Phe Thr Leu Thr Asp Ala Phe 65 70	
Ile Phe Leu Asn Ala Tyr Phe Lys Lys Leu Gly Ser Ser Glu Leu Ala 95 85	

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Tyr Gln Asn Arg Leu Lys Thr Phe Gln Tyr Leu Leu Lys Asn Glu Pro 115

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Cys Glu Tyr Asn Ser Glu Glu Ser Lys Asp His Leu Leu Glu His Met 165 170 175

Glu Asn Arg Phe Asp Val Gln Glu Lys Leu Asp Arg Lys Asp Val Tyr 180 185 190

Tyr Glu Glu Asn Gly Lys Met Val Leu Ile Pro Phe Ser Ile His Asp 195 200 205

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Met His Glu Ile Lys Arg Val Ala Ile Tyr Cys
1 5 10 533

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Ala Glu Glu 191 101 50.	725
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225 220 225	1253
Lys var Gru Asi. 240	1301
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Page 107

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10295.204.ST25.txt

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10295.204.ST25.txt

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Arg Gln Phe Arg Tyr Pro Thr Glu Tyr Gly Gly Gln Lys Pro Gly Thr 150

Ala Thr Ser Thr Val Thr Gly Ser Gly Ala Val Val Leu Ser Gln Gln 170

Pro Gly Gly Ile Lys Ile Thr Ser Ala Thr Val Gly Arg Val Ile Asp 180 180

Leu Gly Ile Thr Asp Ser Gln Asp Met Gly Ser Ala Met Ala Pro Ala 205 195

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Pro Ile Leu Lys Asp Leu Leu Lys Glu Glu Gly Ile Asn Val Gly Thr 255

Lys His Asn Asp Cys Gly Leu Met Ile Tyr Thr Pro Asp Gln Gln Val 260 260

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His Ile Phe Lys Glu Ile Glu Ala Gly Arg Leu Asn Arg Val Leu Val 290

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Bacillus licheniformis

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Glu Asp Ile Val Glu Asn Arg Leu Leu Asn Gln Gln Val Ser Lys Ala 85 90 95

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Ile Gly Glu Arg Ser Lys Thr Asp Ile Cys Leu Cys Tyr Leu Glu Asp 180 180

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Val Lys Ile Asp Gly Leu Pro Met Ser Asp Lys Ser Val Glu Glu Phe 210

Leu Val Gly Gln Gly Tyr Asn Pro Phe Pro Leu Val Arg Phe Thr Glu 235 230 230

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Ile Val Asp Thr Ser Pro Ser Val Ile Ile Thr Pro Thr Thr Leu Phe 265 270

His His Val Gln His Ala Glu Glu Tyr Arg Gln Thr Pro Ala Val Gly 285

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WO 03/087148	CT/DK03/00200
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125 130 130 135	965 ·
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Bacillus licheniformis <213>

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35

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Bacillus licheniformis

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10295.204.ST25.txt Asp Leu Ala Thr Arg Pro Gly Gly Thr Asp Phe Asp Phe Ala Glu Lys 255 Gln Gly Ile Lys Ala Leu Leu Ala Pro Gly Leu Pro Gly Ile Val Ala 260 260 Pro Lys Thr Ala Gly Gln Ile Ile Ala Asn Val Leu Cys Asn Leu Leu 280 285 Ser Glu Leu Thr Thr Asp Arg Lys Gly Leu Ser 290 94 <210> <211> <212> 1588 DNA Bacillus licheniformis <220> CDS <221> (501)..(1088) 60 atacctacag tcgaaggtgc cattatgatg gccatacagc atacagactt tacgattcac 120 ggctcgaatg taatggttct cgggctgggg cggacgggaa tgagcatcag ccggacgttc tcggcgctcg gcgcacgcgt aaaagtcgga gctcgcgact ccgcccacct cgccagaatc 180 atggagatgg gcctcactcc tttccacaca aacgaacttg cagagcatgt tgaaaatatc 240 gacatatgca tcaataccat tccaagcctg attctcgata aacatgtcct ctcacgaatg 300 360 acacccagaa cattaattct cgatttagca acccgtcccg gaggcacaga ttttgatttt gccgaaaagc aaggcattaa agcgctgctt gctccaggac ttcccgggat cgtcgcgcct 420 aaaacggcgg gacagatcat tgccaatgtt ttgtgcaacc ttttgtctga attaacaact 480 gaccgaaagg ggctgtcata atg tcg atc aaa gga aaa aga atc gga ttt ggc Met Ser Ile Lys Gly Lys Arg Ile Gly Phe Gly 10 533 cta acg ggt tca cat tgt acg tat gat gcc gtt ttt ccg cag att gaa Leu Thr Gly Ser His Cys Thr Tyr Asp Ala Val Phe Pro Gln Ile Glu 25 581 gcg ctg atc aac aaa ggg gct gaa gtc aga ccg gtc gtg acg cat act Ala Leu Ile Asn Lys Gly Ala Glu Val Arg Pro Val Val Thr His Thr 30 35 629 aag tcg acg gat aca cgc ttt gga gaa ggg gaa gaa tgg gtc aga Lys Ser Thr Asp Thr Arg Phe Gly Glu Gly Glu Glu Trp Val Arg 45 677 725 aga ata gaa gag ctg act gga ttt gaa gtc att gat tcc att ccg aaa Arg Ile Glu Glu Leu Thr Gly Phe Glu Val Ile Asp Ser Ile Pro Lys 60 65 70 773 gct gag cct ctc ggg ccg aaa aca ccg ctg gac tgc atg gtt Ala Glu Pro Leu Gly Pro Lys Thr Pro Leu Asp Cys Met Val 80 Val Ālā cca ttg acg gga aat tcg atg agc aag ctt gca aac gcc cag acg gac 821 Page 132

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Bacillus licheniformis

<400> 95

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Thr Arg Phe Gly Glu Glu Glu Trp Val Arg Arg Ile Glu Glu Leu 50 60

Thr Gly Phe Glu Val Ile Asp Ser Ile Pro Lys Ala Glu Pro Leu Gly Page 133

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10295.204.ST25.txt 80 65 70 - 75

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Met Ala Ala Lys Asn Val Tyr Phe Ile Pro Phe Gly Gln Asp Asp Pro 145 150 160

Tyr Lys Lys Pro Asn Ser Leu Val Ala Lys Met Asp Leu Leu Val Pro 175

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gta Val	ttt Phe	gtt Val 110	ttt Phe	att Ile	gac Asp	gct Ala	ttc Phe 115	gat Asp	tat Tyr	cag Gln	ctg Leu	aca Thr 120	gat Asp	gcg Ala	agg Arg		869
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480

533

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Phe Lys Gly Asn Pro Gly Thr Gly Lys Thr Thr Val Ala Arg Leu Ile 100 110	
Gly Arg Leu Phe Tyr Glu Met Asn Val Leu Ser Lys Gly His Leu Ile Page 140	

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10295.204.ST25.txt

115

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Thr Asp Ala Leu va. 65	773
Asp Giu vai Tie in 85	821
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Ala Glu Pro Val Phe Ala Asp Val Asp Pro Glu Thr Tyr Asn Leu Asp 105

Pro Lys Lys Ile Glu Glu Lys Ile Thr Pro Ala Thr Lys Ala Ile Ile 115

Pro Val His Ile Phe Gly Gln Pro Ala Asp Met Asp Glu Ile Met Glu 130

Leu Ala Lys Lys His Gly Leu Leu Val Ile Glu Asp Ala Cys Gln Ala 145 150

Phe Gly Ala Ser Tyr Lys Glu Arg Pro Val Gly Ser Ile Gly Asp Ala 175

Ala Cys Phe Ser Phe Phe Pro Thr Lys Asn Leu Gly Thr Leu Gly Asp 180

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Page 144

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175

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165

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Bacillus licheniformis

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Bacillus licheniformis

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Tyr Ala Ser Leu Asn Gln Leu Tyr Glu Asn Ser Lys Asp Ile Gln Asn 130 135

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His Tie Val Ala Lys Cys Gly Ala Thr Tie Ser Lys Met Phe Val Glu aaa ggc gag ccc ctc gtc acg gtg aac cag cag gtt gaa aaa ggg caa Lys Gly Glu Pro Leu Val Thr Val Asn Gln His Val Glu Lys Gly Gln 220 atg ctc gtt tcc ggg ctg atc gga agc gaa gag gaa aag caa aaa gtc Met Leu Val Ser Gly Leu Ile Gly Ser Glu Glu Glu Lys Gln Lys Val 225 gga gca aaa ggg aaa atc tat ggt gaa acc tgg tac aag tca aca gta Gly Ala Lys Gly Lys Ile Tyr Gly Glu Thr Trp Tyr Lys Ser Thr Val 245 acg gtt cct cttt gag aca tca ttt gac gtt ttt acg ggt aaa gta agc agg Thr Val Pro Leu Glu Thr Ser Phe Asp Val Phe Thr Gly Lys Val Arg 255 aca agt cac aag cta tcc ctc gga tca tta acc atg ccg atc tgg ggc Thr Ser His Lys Leu Ser Leu Gly Ser Leu Gly Ser Arg Pro Lys Thr Glu 276 aca aca cac tcg ctg cat ttt ata aat ttt aag ctc ctc gu aag acg gaa acc ctc gga acc acc acc acc acc gga Thr Ser Phe Lys Lys Glu Asp Phe Ser Arg Pro Lys Thr Glu 280 aaa cac tcg ctg cat ttt ata aat ttt aag ctt ctc ttt gag acc gaa Lys His Ser Leu His Phe Ile Asn Phe Lys Leu Pro Val Ala Tyr Glu 305 aaa gaa gaa gac att cta aga agg acc acc acc acc acc acc acc gaa Lys Glu His Met Arg Glu Ser Glu Gln Tie Lys Arg Val Tyr Ser Lys 326 aaa gaa gaa gca gtt ctt aga agg agc gaa cat tat caa acc gg acc acc acc acc acc acc acc	cac	ato	· atc	acc	222	222	aaa							ttc	ata	паа		866
atg ctc gtt tcc ggg ctg atc ggy gaa gac gaa gaa gaa gaa gaa gaa gaa gaa	His	Ile	Val	Ala	Lys	Lys	Gly	Ala	Thr	Ile	Ser	Lys	Met	Phe	Va]	Glu		000
Met Leu Val Ser člý Leŭ Ile člý sěr člu člu člá člu Lyš cln Lys Val 235 cln Lys Val 225 cln Lys Val 225 cln Lys Val 235 cln Lys Val 235 cln Lys Val 235 cln Lys Val 235 cln Lys Val 240 cln Z45 cln Z45 cln Z46 cln Z4	aaa Lys	ggc Gly	gag Glu	Pro	ctc Leu	gtc Val	acg Thr	gtg Val	Asn	cag Gln	cac His	gtt Val	gaa Glu	Lys	ggg Gly	caa Gln		914
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Thr Val Pro Leu Glü Thr Ser Phe Asp Val Phe Thr Gly Lys Val Arg 270 aca agt cac aag cta tcc ctc gga tca tta acc atg ccg atc tgg ggc Thr Ser His Lys Leu Ser Leu Gly Ser Leu Thr Met Pro Ile Trp Gly 285 ttt tca ttt aaa aaa gaa gac ttc tcg cgc ccg aag acg gag acc gaa Phe Ser Phe Lys Lys Glu Asp Phe Ser Arg Pro Lys Thr Glu Thr Glu 300 aaa cac tcg ctg cat ttt ata aat ttt aag ctt cct gtc gct tat gaa Lys His Ser Leu His Phe Ile Asn Phe Lys Leu Pro Val Ala Tyr Glu 310 aag gag cat atg agg gag agc gaa caa atc aaa agg gtg tac tcg aaa Lys Glu His Met Arg Glu Ser Glu Gln Ile Lys Arg Arg Val Tyr Ser Lys 320 aaa gaa gca gtt ctt aga agg aat cga aat ggg aaa aag aag agg cat cag Lys Glu Ala Val Leu Arg Arg Asn Arg Asn Gly Lys Lys Arg His Gln 350 gac aaa aat cgg cag aga cgg gaa cat tat cag tgt aaa agt ttt gca Asp Lys Asn Arg Gln Arg Arg Glu His Tyr Gln Cys Lys Ser Phe Ala 365 cac cac gag His His Glu 		Āla	Lys				Tyr					Tyr						1010
Thr Ser His Lys Leu Ser Leu Gly Ser Leu Thr Met Pro Ile Trp Gly 285 ttt tca ttt aaa aaa gaa gac ttc tcg cgc ccg aag acg gag acc gaa Phe Ser Phe Lys Lys Glu Asp Phe Ser Arg Pro Lys Thr Glu Thr Glu 290 aaa cac tcg ctg cat ttt ata aat ttt aag ctt cct gtc gct tat gaa Lys His Ser Leu His Phe Ile Asn Phe Lys Leu Pro Val Ala Tyr Glu 315 aag gag cat atg agg gag agc gaa caa atc aaa agg gtg tac tcg aaa Lys Glu His Met Arg Glu Ser Glu Gln Ile Lys Arg Val Tyr Ser Lys 320 aaa gaa gca gtt ctt aga agg aat cga aat ggg aaa aag agg cat cag Lys Glu Ala Val Leu Arg Arg Asn Arg Asn Gly Lys Lys Arg His Gln 350 gac aaa aat cgg cag aga cgg gaa cat tat cag tgt aaa agt ttt gca Asp Lys Asn Arg Glu His Tyr Gln Cys Lys Ser Phe Ala 365 cac cac gag His His Glu 2210> 121 2210> 121 2210> 121 2210> PRT	Thr	gtt Val	cct Pro	ctt Leu	gag Glu	Thr	tca Ser	ttt Phe	gac Asp	gtt Val	Phe	acg Thr	ggt Gly	aaa Lys	gta Val	Arg		1058
Phe Ser Phe Lys Lys Glu Asp Phe Ser Arg Pro Lys Thr Glu 300 Thr Glu 295 aaa cac tcg ctg cat ttt ata aat ttt aag ctt cct gtc gct tat gaa Lys His Ser Leu His Phe Ile Asn Phe Lys Leu Pro Val Ala Tyr Glu 310 aag gag cat atg agg gag agc gaa caa atc aaa agg gtg tac tcg aaa Lys Glu His Met Arg Glu Ser Glu Gln Ile Lys Arg Val Tyr Ser Lys 320 aaa gaa gca gtt ctt aga agg aat cga aat ggg aaa aag aga cat cag Lys Glu Ala Val Leu Arg Arg Asn Arg Asn Gly Lys Lys Arg His Gln 350 gac aaa aat cgg cag aga cgg gaa cat tat cag tgt aaa agt ttt gca Asp Lys Asn Arg Gln Arg Arg Glu His Tyr Gln Cys Lys Ser Phe Ala 355 cac cac gag His His Glu					Leu					Leu					Trp			1106
Lys His Ser Leu His Phe Ile Asn Phe Lys Leu Pro Val Ala Tyr Glu 305 aag gag cat atg agg gag agc gaa caa atc aaa agg gtg tac tcg aaa Lys Glu His Met Arg Glu Ser Glu Gln Ile Lys Arg Val Tyr Ser Lys 320 aaa gaa gca gtt ctt aga agg aat cga aat ggg aaa aag aga cat cag Lys Glu Ala Val Leu Arg Arg Asn Arg Asn Gly Lys Lys Arg His Gln 335 gac aaa aat cgg cag aga cgg gaa cat tat cag tgt aaa agt ttt gca Asp Lys Asn Arg Gln Arg Arg Glu His Tyr Gln Cys Lys Ser Phe Ala 355 cac cac gag His His Glu	ttt Phe	tca Ser	ttt Phe	Lys	Lys	gaa Glu	gac Asp	ttc Phe	Ser	cgc Arg	ccg Pro	aag Lys	acg Thr	Glu	acc Thr	gaa Glu		1154
Lys Glu His Met Arg Glu Ser Glu Gln Ile Lys Arg Val Tyr Ser Lys 320 aaa gaa gca gtt ctt aga agg aat cga aat ggg aaa aag aga cat cag Lys Glu Ala Val Leu Arg Arg Asn Arg Asn Gly Lys Lys Arg His Gln 335 gac aaa aat cgg cag aga cgg gaa cat tat cag tgt aaa agt ttt gca Asp Lys Asn Arg Gln Arg Arg Glu His Tyr Gln Cys Lys Ser Phe Ala 355 cac cac gag His His Glu <pre></pre>			Ser					Asn					٧a٦				•	1202
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·	<211 <212	1> 2>	369 PRT	llus	lic	henii	form t	is										
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Ile Thr Gly Lys Gly Ile Glu Arg Leu Leu Asn Glu Cys Thr Arg Arg 20 25 30	Ile	Thr	Gly	Lys 20	Gly	Ile	Glu	Arg		Leu	Asn	Glu	Cys		Arg	Arg		

PCT/DK03/00200

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Asn Ile Pro Met Phe Asn Val Lys Lys Lys Lys Asp Ala Val Phe Leu 35 40 45

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10295.204.ST25.txt

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Phe Phe Ile Met Phe Leu Leu Ser Asn Met Leu Trp Lys Ile Asp 100 100 110

Ile Thr Gly Ala Asn Pro Glu Thr Glu His Gln Ile Arg Gln Gln Leu 125 115

Asp Gln Ile Gly Val Lys Lys Gly Arg Phe Gln Phe Ser Met Leu Thr 130

Pro Glu Lys Ile Gln Gln Ala Leu Thr Lys Arg Val Glu Asn Ile Thr 145 150 160

Trp Val Gly Ile Glu Leu Asn Gly Thr Ala Leu His Met Lys Val Val 170 175

Glu Lys Asn Glu Pro Asp Lys Glu Lys Tyr Ile Gly Pro Arg His Ile 180 180

Val Ala Lys Lys Gly Ala Thr Ile Ser Lys Met Phe Val Glu Lys Gly 195 200

Glu Pro Leu Val Thr Val Asn Gln His Val Glu Lys Gly Gln Met Leu 210

Val Ser Gly Leu Ile Gly Ser Glu Glu Glu Lys Gln Lys Val Gly Ala 230 235

Lys Gly Lys Ile Tyr Gly Glu Thr Trp Tyr Lys Ser Thr Val Thr Val 255 245

Pro Leu Glu Thr Ser Phe Asp Val Phe Thr Gly Lys Val Arg Thr Ser 260 265

His Lys Leu Ser Leu Gly Ser Leu Thr Met Pro Ile Trp Gly Phe Ser 285

Phe Lys Lys Glu Asp Phe Ser Arg Pro Lys Thr Glu Thr Glu Lys His 290

Ser Leu His Phe Ile Asn Phe Lys Leu Pro Val Ala Tyr Glu Lys Glu 315 320 10295.204.ST25.txt His Met Arg Glu Ser Glu Gln Ile Lys Arg Val Tyr Ser Lys Lys Glu 325 330 335

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aag cag aat atg gaa aga ctg tct gac att ctg aac ata ccc gaa ccg Lys Gln Asn Met Glu Arg Leu Ser Asp Ile Leu Asn Ile Pro Glu Pro 15 20 25	581
ctt tta atc agt gca aat gca aat gta tcc gcg gac gaa ctt tat ttt Leu Leu Ile Ser Ala Asn Ala Asn Val Ser Ala Asp Glu Leu Tyr Phe 30 35	629
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WO 03/08/140	
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110 115	917
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gat att ttt tca tca aca aag ctt tcc gtc gtg ccg atc gtt aat ccc Asp Ile Phe Ser Ser Thr Lys Leu Ser Val Val Pro Ile Val Asn Pro 180	1061
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220 225 225	1253
Phe Trp Giu 116 340 245	1301
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atg agg gat tta atc gca aac gag ccg cct gac cgg ctt gtg gcg ctt Met Arg Asp Leu Ile Ala Asn Glu Pro Pro Asp Arg Leu Val Ala Leu Met Arg Asp Leu Ile Ala Asn 275	1349
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Lys Gly val Alg 320	1541
Tile His Tyling 1975	1589
aaa aat cct tta ccg ctg aaa caa ttt gac gat ata tat tgt aaa agc Lys Asn Pro Leu Pro Leu Lys Gln Phe Asp Asp Ile Tyr Cys Lys Ser Lys Asn 350	•
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10295.204.ST25.txt Arg Gly Ile Leu Trp Ala Ser Cys Phe Phe Glu Ser 365 370 375

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		accgttatat				1935
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His Ala Gly Lys Asn Val Gln Ala Ala Glu Thr Tyr Glu Gln Leu Gln 50 55 60

Leu Leu Ala Asn Gln Tyr Thr Phe Glu Asp Glu Gln Trp Leu Thr Lys 65 70 75

Thr Ala Val Tyr Asp Ser Ala Glu Leu Lys Lys Glu Ile Gly Arg Leu 85 90 95

Thr Glu Cys Phe Pro Phe Val Thr Ser Arg Ile Ile Gly Arg Ser Ser 100 105 110

Met Gly Gln Pro Ile Tyr Glu Leu Leu Leu Gly Ala Glu Asn Ala Gly 115 120 125

Lys Arg Thr His Met Asn Ala Ser Phe His Ala Asn Glu Trp Ile Thr 130 140

Thr Ser Val Leu Met Lys Trp Leu Lys Glu Tyr Cys Tyr His Leu Cys 145 150 150

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Thr Gly Gln Thr Ala Leu Gly Phe Ser Pro Leu Asp Ile Phe Ser Ser 175

Thr Lys Leu Ser Val Val Pro Ile Val Asn Pro Asp Gly Val Asp Leu 180 180

Val Leu Asn Gly Pro Gly His Leu Gly Ile Ala Arg Glu Ala Leu Asp 195

Glu Met Asn Glu His Gln Pro Asp Phe Arg Glu Trp Lys Ala Asn Ile 210 220

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Lys Gln Arg Lys Pro Pro Lys Ser Pro Ser Tyr Arg Asp Tyr Pro Gly 255

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Ala Asn Glu Pro Pro Asp Arg Leu Val Ala Leu His Thr Gln Gly Glu 280 285

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· Val Ile Gln Thr Phe Glu Arg Leu Ser Gly Tyr Lys Gly Val Arg Tyr 320 305

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Pro -																
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Glu I	Leu	Leu	Met	G]u 165	Ala	Asp	Ile	Asp	∨a1 170	Arg	Asp	Ile	Leu	Glu 175	Glu	
Asp /	Asp	Ala	va1 180	Ile	val	Tyr	Ala	Glu 185	Pro	Asp	Gln	Phe	ніs 190	Ala	Val	
Gln	Glu	Ala 195	Leu	Gln	Asn	Αla	Gly 200	Ile	Thr	Glu	Phe	Thr 205	val	Ala	Glu	
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			Ala A	Ala /	5				(SP)	Asp A	ĀΊa Α	Ārg -	Thr (ыу.	11e 15	
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Ala Leu Lys Flo Ala 150	527
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370 Page 182	

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Phe Ser Leu Phe Thr Val Asn Ala Ala Ile Leu Gln Gly Val Asn Lys 305 310 315

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Phe Leu Leu Met Leu Ile Leu Thr Ala Val Met Gly Ile Ile Leu Leu

Leu Val Gln Ala Leu Leu Ser Ile Phe Ile Ser Tyr Glu Gly Gln 405 410 415

Ile Arg Ser Ala Val Val Ile Phe Ile Thr Thr Ala Val Gly Gly Ser 420 425 430

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cag ccg cgc acc att ttc tca gaa gaa aaa att aaa gaa tta gct gca Gln Pro Arg Thr Ile Phe Ser Glu Glu Lys Ile Lys Glu Leu Ala Ala 677 Page 185

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Phe Ser Glu Glu Lys Ile Lys Glu Leu Ala Ala Thr Ile His Thr His 50 60

Gly Ile Ile Gln Pro Ile Val Val Arg Lys Thr Glu Arg Glu Gly Gln 65 70 75

Tyr Glu Leu Ile Ala Gly Glu Arg Arg Trp Arg Ala Val Gln Thr Leu 85 90 95

Asp Trp Glu Lys Val Pro Ala Ile Ile Lys Asp Phe Ser Asp Thr Glu 100 105 110

Thr Ala Ser Val Ala Leu Ile Glu Asn Leu Gln Arg Glu Glu Leu Ser 115 120 125

Ser Ile Glu Glu Ala His Ala Tyr Ala Arg Leu Leu Glu Leu His Asp 130 140

Leu Thr Gln Glu Ala Leu Ala Gln Arg Leu Gly Lys Gly Gln Ser Thr 145 150 160

Ile Ala Asn Lys Leu Arg Leu Leu Lys Leu Pro Glu Glu Val Gln Glu 165 170 175

Ala Ile Leu Lys Lys Glu Ile Ser Glu Arg His Ala Arg Ala Leu Ile 180 185

Pro Leu Lys Gln Pro Asp Leu Gln Val Lys Leu Leu His Glu Val Ile 195 200 205

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Gln Ser Ala Asn Gly Glu Pro Val Ala Val Leu His Gly Asp Glu Val 35 40 45

Arg Leu Ile Ala Asp Ala His Leu Gly Asp Leu Glu Ile Val Arg Glu 50 60

Ala Glu Trp Gln Met Arg Lys Arg Glu Glu Glu Thr Arg Met Lys Glu 65 70 75 80

Ser Leu Asp Leu Arg Gln Asp Tyr Lys Leu Leu His Asp Lys His 85 90 · 95

Glu Tyr Arg Ala Thr Asn Gln Tyr Asn Asn Gln Gln Gln Tyr Phe His 100 105 110

Met Pro Gly Arg Val Leu His Leu Asp Gly Asp Ser Ala Tyr Leu Lys 115 120 125

Lys Cys Leu Ala Leu Tyr Glu Lys Ile Gly Val Pro Val Tyr Gly Ile 130 140

His Cys Tyr Glu Lys Lys Met Ser Ser Val Ile Glu Glu Leu Ile Asp 145 150 160

Glu Tyr Arg Pro Asp Leu Leu Val Ile Thr Gly His Asp Ala Tyr Ser 165 170 175

Lys Gln Lys Gly Asp Ile Asn Asn Leu Asp Ala Tyr Arg His Ser Lys 180 185 ... 190

Asp Phe Ile Glu Thr Val Gln Lys Ala Arg Arg Lys Ile Pro His Leu 195 200 205

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02007440A2 1 -

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WO 03/087148 PCT/DK03/00200

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Pro Thr Arg Tyr Ser His Trp Ser Phe Gly Lys Gln Phe His Lys Met 50

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Asn Ser Asp Pro Cys Tyr Ala Phe Leu Leu Asp Ser Asn Ser Leu Ile 85 90 95

Gln Asn Lys Leu Ile Val Ala His Val Leu Ala His Cys Asp Phe Phe 100 105 110

Lys Asn Asn Cys Arg Phe Gln Asn Thr Lys Arg Asp Met Val Glu Ser 115 120 125

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Ser Lys Glu Val Glu Ala Phe Leu Asp Ala Val Leu Ala Ile Glu Glu 145 150 150

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Leu Trp Glu Leu Asp His Lys Gly Ser Lys Glu Lys Lys Lys Arg Thr 195 200 205

Lys Lys Lys Phe Pro Pro Lys Pro Glu Lys Asp Ile Leu Leu Phe Ile 210 215

Glu Glu His Ser Arg Glu Leu Glu Pro Trp Gln Arg Asp Ile Leu Thr 235 240 Page 197

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Phe	Lys	Lys	Ile	Arg 85	Asp	Gln	Asn	Asn	Leu 90	Glu	Gly	Met	Leu	Asp 95	Tyr
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Pho	e Asp 130	cys)	s Asp	o Glu	Leu	G]u 135	Lys	: Ala	. Glu	ı Phe	Туг 140	Phe	Lys	. Ala	ser
G]1	u Va ⁻ 5	І Туі	туі	r His	Met 150	Lys	G]r	Thr	· Ile	Phe 155	Ser	Met	. Asr	ı Tyr	160
Se	r Ar	g Ala	а Ту	r Ası 16!	ı Leu	Phe	e Lys	s Lys	5 Tyr 17(Asp O	Thr	Туі	r Gly	/ Glu 175	ı Arg
Ar	g Va] G]	n Se 18	r Gli O	n Phe	e Il	e Ile	e Ala 18	a Gly 5	y Asr	1 Trp) Lei	u Ası 19	р His	s Met
Ту	r Pr	o Gl 19	u Ly 5	s Al	a Lei	ı Ні	s Ası 20	n Le	u Asi	n Ly:	s Glu	ı Le 20	u Ly 5	s G]	u Ser
G٦	u Th 21	r G7 .0	n Gl	y Il	e Le	и Ні 21	s Le 5	u Me	t Gl	y se	r Sei 220	r Hi O	s Le	u As	n Ile
G] 22	y Il 25	e Cy	's Ty	r As	n Ly 23	s Le O	u Gl	u As	p Va	1 As 23	р Ly: 5	s Al	a Th	г Ту	r Asn 240

WO 03/087148 PCT/DK03/00200

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Leu S	ser	11e 275	Thr	Asp	Asp	Leu	туг 280	туг	Glu	Gly	Lys	G]u 285	Leu	Ala	Glu	
Lys /	4sn 290	Lys	Asn	Leu	Asp	Met 295	Leu	Ala	Lys	Phe	Asp 300	Leu	Ile	Lys	Gly	
Leu ⁻ 305	Tyr	Leu	ser	Phe	Asp 310	Leu	Asp	Met	val	Arg. 315	Glu	Ser	Phe	Lys	Phe 320	
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Ala	Αla	Glu	Leu 340	Leu	Glu	Lys	Lys	G]u 345	Lys	Ile	Arg	Asp	А1а 350	val	Glu	·
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٧a	l Va	27	s Gli O	ı Ala	a Arg	ı Lys	275	ilyi	GIU	Lys		280)	- A' S	g gcg g Ala	1349
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gc Al 30	a Le	g ta u Ty	t ga r As	t gaa p Glu	a cag u Glr 305	ı Asp	cca Pro	ctt Lei	aco Thr	gti Va 310	GIL	a cat u His	gct s Ala	t tta a Lei	a gaa u Glu 315	1445
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Page 212

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Glu Glu Phe Ala Ala Ser Asn Lys Leu Phe Glu Asp Ile Gln Glu Gln 65 70 75

Lys Ala Asp Met Gln Ser Thr Asp Asp Met Ile Glu Tyr Tyr Tyr Phe 85 90 95

Phe Phe Ala Gly Met Tyr Glu Phe His Lys Lys Asp Tyr Thr Asn Ala 100 100 110

Ile Asn Tyr Tyr Lys Leu Ala Glu Glu Lys Leu Arg Thr Ile Pro Asp 115 120 125

Gln Ile Glu Ile Ala Glu Phe His Tyr Lys Leu Ala Ile Ala Tyr Tyr 130 135 140

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His His Val Leu Leu Gly Met Ala His His Asn Leu Gly Leu Ser Tyr 210 215 220

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PCT/DK03/00200 WO 03/087148

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ga [.] Ası	t tc p Se	c ata r Il 350	e Phe	t ttt e Phe	tat Tyi	gat Asp	cag Glr 355	I ME	. va	GII	ı Alc	cag Glr 360	· -y ·	a caa s Gli	a atc n Ile	1589
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773

821

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Pro 65	Ser	Arg	val	Asp	Phe 70	Met	: Pr	o Le	u Hi	s Ar 75	g Le	u Il	e Th	r Gl	u G	llu O
Glu	۷al	Asp	Asp	∨a1 85	۷a٦	нis	ra :	a Va	را ل 90	/s As)	p Va	l Le	u Pr	o Th 95	ır G	IJ
G]n	Phe	Thr	Ser 100	Gly	Phe	Туг	· va	1 G] 10	y Va)5	al Ph	ie Gl	u Al	a G]	u I LO	le A	ala

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PCT/DK03/00200

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WO 03/087148 PCT/DK03/00200

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160

10295.204.ST25.txt 155

145 150⁻¹

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WO 03/087148

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gat atg tcc ggg ctg ttt aaa atc att cgc ctt gag caa tca Asp Met Ser Gly Leu Phe Lys Ile Ile Arg Leu Glu Gln Ser 95 100 105	gag cag Glu Gln
cgt gca ctt gaa acg ttg ggg gtg gcg tca tgaaaaatga aatga Arg Ala Leu Glu Thr Leu Gly Val Ala Ser 110 115	aacatt
cagtttacag cgctcagcca aaatgaatcg tttgcacggg tgacagtcgc	tgcttttatc
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Asn Leu Ala Asp Leu Ser Phe Met Asp Ser Ser Gly Leu Gly 50 60	y Val Val
Leu Gly Arg Tyr Lys Glu Ile Lys Gln Leu Gly Gly Glu Met 65 70 75	t Ile Val 80
Cys Ala Ile Ser Pro Ala Val Lys Arg Leu Phe Asp Met Ser 85	r Gly Leu 95
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gat tca ttc ttt gga gaa aaa ggt acg ttc tct ttc atc cgc ctc gac Asp Ser Phe Phe Gly Glu Lys Gly Thr Phe Ser Phe Ile Arg Leu Asp 125	917
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. 23	

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Page 251	

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Page 253	

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ga G1	g tto u Phe 142	e Tr	a aa ir Ly	a gg	c tar y Tyr	t gat r Asp 143	_ ၁૯	g ct r Le	g ac u Th	a ag r se	c gaa r Glu 143	_	c aa l Ly	g cag s Gln	4814
gt Va	c aga 1 Arg 144	ь са у Н ¹ 10	is Al	g at a Me	g cta t Le	a ttg u Leu 144	at Me 5	g aa t Ly	a aa s Ly	a tc s Se	c gag r Glu 145	ca Gl	g aa n As	c ttg n Leu	4859
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Glu Thr Val Ser Ser Leu Lys Leu Gly Gly Lys Ala Ser Val Gln Ser 65 70 75

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150

10295.204.ST25.txt 155

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Glu Gly Pro Met Gly Val Val Gly Lys Leu Ser Val Val Lys Asn Glu Page 256

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Gly Val Ser Thr Ile Val Ala Ala Glu Thr Lys Glu Ser Leu Ser Glu 545 550 560

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10295.204.ST25.txt 695 700

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975

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Gly Ser Ser Gly Tyr Gly Lys Ser Leu Thr Ala Thr Thr Phe Met 1010 1020

Met Ser Phe Ala Glu Gln Tyr Thr Pro Glu Glu Leu His Tyr Tyr 1025 1030 1035

Ile Phe Asp Phe Gly Asn Gly Thr Leu Leu Pro Leu Ala Arg Leu 1040 1050

Pro His Thr Ala Asp Tyr Phe Leu Met Asp Gln Thr Arg Lys Ile 1055 1060 1065

Glu Lys Phe Met Val Arg Ile Lys Ala Glu Ile Glu His Arg Lys 1070 1080

Asn Leu Phe Arg Ala Lys Glu Ile Ser His Ile Lys Met Tyr Asn 1085 1090 1095

Ala Leu Asn Glu Glu Lys Leu Pro Phe Ile Phe Ile Thr Val Asp 1100 1105

Asn Phe Asp Ile Ile Lys Asp Glu Met His Glu Leu Glu Ser Glu 1115 1120 1125

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Ile Pro Gly Arg Val Ile Ile Asn Lys Glu Asn Gln Tyr Phe Ala 1190 1200

Gln Met Phe Met Pro Val Glu Ala Asp Asn Asp Ile Glu Leu Phe 1205 1210

Glu Gly Ile Lys Ala Asp Ile Gln Ala Ile Ala Glu Arg Ser Glu Page 259 10295.204.ST25.txt 1220 1225 1230

Gly Met Arg Lys Pro Ala Pro Val Pro Met Leu Pro Leu Glu Leu 1235 1240 1245

Ser Val Thr Gln Phe Val Arg Asp Tyr Pro Leu Gln Pro Glu Arg 1250 1260

Gly Leu Ile Pro Met Gly Leu Asp Glu Glu Thr Val Glu Pro Val 1265 1270 1275

Tyr Phe Asn Leu Glu Lys Asn Lys His Cys Leu Ile Met Gly Gln 1280 1290

Thr Gln Arg Gly Lys Thr Asn Val Ile Lys Ile Met Leu Glu His 1295 1300 1305

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Val Asp Gly Ile Ser Arg Phe Gln Gln Thr Ile Asp Ala Ser Ile 1385 1390 1395

Gln Asp Lys Met Ala Met Phe Met Lys Ser Tyr Ala His Leu Gly 1400 1405 1410

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Glu Arg Gln Glu Pro Glu Ile Leu Pro Gly Phe Gly Tyr Ile Val 1460 1465 1470

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tac tgc atc att gca agc atg ctg ctt ctt tca cat gtc cag ctg ttt Tyr Cys Ile Ile Ala Ser Met Leu Leu Ser His Val Gln Leu Phe cag cat ttg acc gaa agg gga atg gtt cag tct ccg agc gtg atc caa Gln His Leu Thr Glu Arg Gly Met Val Gln Ser Pro Ser Val Ile Gln 869

85

Trp Lys Lys Lys Thr Pro Ser Phe Leu Thr Arg Arg Lys Ala Gly Leu

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	125					130	1	.0295	.204	ST2	135 135	ťτ				
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tat Tyr	ttt Phe	ctg Leu	ttt Phe	gca Ala 160	tct Ser	gca Ala	gga Gly	tct Ser	aaa Lys 165	atc Ile	atc Ile	gcc Ala	gtc Val	ttc Phe 170	ctg Leu	1013
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Se 38	r As O	p As	p Le	u Ai	a Le	u A11	a Le	u Al	a Ali	39 ¹	0	J 1.1.	C //()	.	c gaa e Glu 395	1685
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Gli	1 Thi	- AS	sei	640)	. Т16	e rei	. Ast	64	5	<i>,</i>	y		65		2453
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n Leu Gly Val Ala Gly Gl
n Thr Phe $35 \ \ \,$

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Leu Lys Thr Ile Gly Asp Gln Thr Phe Asp Arg Asn Tyr Gln Arg Ile 130 135

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725

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Page 270

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276	

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Met Phe Phe Ile Met Asn Val Asp Tyr Trp Thr Trp Arg Thr Tyr Ala 65 70 75

Lys Ile Leu Ile Ile Val Cys Phe Phe Leu Leu Ile Ile Val Leu Val 85 90 95

Pro Gly Ile Gly Met Glu Arg Asn Gly Ser Arg Ser Trp Ile Gly Val 100 105 110

Gly Ala Phe Ser Ile Gln Pro Ser Glu Phe Met Lys Leu Ala Met Ile 115 120 125

Ala Phe Leu Ala Lys Phe Leu Ser Glu Lys Gln Lys Asn Ile Thr Ser 130 140

Phe Arg Lys Gly Phe Val Pro Ala Leu Gly Ile Val Phe Ser Ala Phe 145 150 155 - 160

Leu Ile Ile Met Met Gln Pro Asp Leu Gly Thr Gly Thr Val Met Val 165 170 175

Gly Thr Cys Ile Ile Met Ile Phe Val Ala Gly Ala Arg Ile Ser His 180 185

Phe Val Phe Leu Gly Leu Ile Gly Leu Ser Gly Phe Val Gly Leu Val 195 200 205

Leu Ser Ala Pro Tyr Arg Ile Lys Arg Ile Thr Ser Tyr Leu Asn Pro 210 215 220

Trp Glu Asp Pro Leu Gly Ser Gly Phe Gln Ile Ile Gln Ser Leu Tyr 225 230 240

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DECONO - WILL 0208714842 |

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·	·				
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Phe His Glu Ala Ala Glu Ile Gly Thr Arg Lys Val Ile Gln Glu His 130 140

Ser Thr Ile Gly Val Val Ile Thr Thr Asp Gly Thr Ile Gly Glu Ile 145 150 150

Ala Arg Gln Asp Tyr Val Glu Ala Glu Glu Arg Val Ile Asp Glu Leu 165 170 175

Lys Glu Val Gly Lys Pro Phe Ile Met Val Ile Asn Ser Val Arg Pro 180 185 190

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Val Asn Val Asn Leu Pro Ser Trp Val Met Val Leu Lys Glu Asn His 245 250 255

Trp Leu Arg Glu Asn Tyr Gln Asp Ser Val Lys Glu Thr Val Lys Asp 260 265 270

Ile Lys Arg Leu Arg Asp Val Asp Arg Val Val Gly His Phe Ser Glu 275 280 285

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Ala Pro Ala Leu Thr Asp Met Ser Leu Asp Glu Pro Glu Ile Ile Arg 370 375 380

Gln Gly Ser Arg Phe Gly Val Arg Leu Lys Ala Val Ala Pro Ser Ile 385 390 395 400

His Met Ile Lys Val Asp Val Glu Ser Glu Phe Ala Pro Ile Ile Gly
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Thr Glu Lys Gln Ser Glu Glu Leu Val Arg Tyr Leu Met Gln Asp Phe 420 425 430

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gaa gaa gaa gaa gat cct gtc ctg aat gac gaa aat gaa gat gaa att tat ttg gaa aat gaa gaa	Val Gin Led Gly Gly Glu IIII Val Sci. 30 70 75	725
gaa gac gac agc gtt cct gcc tac tat ttg gga gag ag gil gil lie ser ser Leu Asp Gln Glu Ala Asp Ser Phe Phe Gly Glu Asp Gln Glu Asp Val Tyr Phe Ash Glu Thr 120 gat tca ttc ttc gga gaa aaa ggt acg ttc tct tct act cgc ctc gac Asp Ser Phe Phe Gly Glu Lys Gly Thr Phe Ser Phe Ile Arg Leu Asp 135 ggg caa tgg aat gtc ctg ccg aat gac gcg aaa att tat ttg aac gga gag gag gaa aat ggc ggg gaa att tat ttg aac gga gac gal gly Glu Trp Ash Val Leu Pro Ash Asp Ala Lys Ile Tyr Leu Ash Gly 155 gaa gaa gaa gtg tcc gcc cct gtc tcc gtg caa aat gga gac gaa atc gca Glu Glu Val Ser Ala Pro Val Ser Val Glu Ash Gly Asp Glu Ile Ala 165 ttt gga ctg aat att ctt cgc atc gtt gaa gac gac gac ctc ttg gaa atc glu Ile Ala 170 ttt gga ctg aat att ctt cgc atc gtt gaa gac gac ctc ttg gaa atc glu Ile Ala 180 gag gga ttc ggg aag ttt gat acg tct ttg gaa gac gac ctc ttg gaa atc glu Ile Ins 185 gag gga ttc ggg aag ttt gat acg tct ttg gag aac att ctt aag ccg Ilos 190 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg ccg aga Ser Ser Glu Thr Lys Ash Lys Tyr Pro Gln Tyr Arg Arg Arg Pro Pro Arg 215 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 225 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 225 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 225 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 225	Ala Ser val Gin Ser Giy Ala Gid Gin Ser Go	773
yal lle ser ser Leu Asp Gln Glu Ala Asp Val Tyr Phe Asr Glu Thr 110 gat tca ttc ttt gga gaa aaa ggt acg ttc tct ttc atc cgc ctc gac Asp Ser Phe Phe Gly Glu Lys Gly Thr Phe Ser Phe Ile Arg Leu Asp 135 ggg caa tgg aat gtc ctg ccg aat gac gcg aaa att tat ttg aac gga Gly Gln Trp Asn Val Leu Pro Asn Asp Ala Lys Ile Tyr Leu Asn Gly 155 gaa gaa gtg tcc gcc cct gtc tcc gtg caa aat gga gac gaa atc gca Glu Glu Val Ser Ala Pro Val Ser Val Gln Asn Gly Asp Glu Ile Ala 170 ttt gga ctg aat att ctt cgc atc gtg gaa gac gac gac ctc ttg gaa atc phe Gly Leu Asn Ile Leu Arg Ile Val Glu Asp Asp Leu Leu Glu Ile 185 gag gga ttc ggg aag ttt gat acg tct ttg gaa gac gac ctc ttg gaa atc gcg Glu Glu Glu Phe Gly Lys Phe Asp Thr Ser Leu Glu Asn Ile Leu Lys Pro 195 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg ccg aga Ser Ser Glu Thr Lys Asn Lys Tyr Pro Gln Tyr Arg Arg Pro Pro Arg 215 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca Phe Ser Phe Pro Ala 235 225	Glu Ala Asp Ser Val Pro Ala 191 190	821
gat tca ttc ttt gga gaa aaa ggt acg ttc tct atc atc aag gga gac gaa atc gga gaa gaa gga gaa gga gaa atc Leu Pro Asn Asp Ala Lys Ile Tyr Leu Asn Gly 155 gaa gaa gtg tcc gcc cct gtc tcc gtg caa aat gga gga gac gaa atc gca lib Ile Arg Leu Asn Gly 155 gaa gaa gtg tcc gcc cct gtc tcc gtg caa aat gga gac gaa atc gca Ile Ala 160 ttt gga ctg aat att ctt cgc atc gtt gaa gac gac ctc ttg gaa atc leu Asn Ile Leu Arg Ile Val Glu Asp Asp Leu Leu Glu Ile 185 gag gga ttc ggg aag ttt gat acg tct ttg gaa gac gac atc ctt ttg gaa atc leu Glu Ile 185 gag gga ttc ggg aag ttt gat acg tct ttg gag aac atc ctt aag ccg Glu Gly Phe Gly Lys Phe Asp Thr Ser Leu Glu Asn Ile Leu Lys Pro 195 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg agg acg acg acg acg acg a	val Tie Ser Ser Leu Asp Gill did Aid Asp Va. 120	869
ggg caa tgg aat gtc ctg ccg aat gac gcg gac aat gga gac gaa atc gca glu Glu Val Ser Ala Pro Val Ser Val Gln Asn Gly Asp Glu Ile Ala 160 ttt gga ctg aat att ctt cgc atc gtt val Glu Asp Asp Asp Leu Leu Glu Ile 185 gag gga ttc ggg aag ttt gat acg tct ttg gag aac att ctt aag ccg Glu Gly Phe Gly Lys Phe Asp Thr Ser Leu Glu Asn Ile Leu Lys Pro 195 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg ccg aga gac gac ctc ttg gag aac att ctt aag ccg 1109 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg ccg aga 1157 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1209	Asp Ser Phe Phe Gly Glu Lys Gly III 112 301	917
ttt gga ctg aat att ctt cgc atc gtt gaa gac gac ctc ttg gaa atc he gga gga gga gga gaa gac gac ctc ttg gaa atc gfu she gly Leu Asn Ile Leu Arg Ile val glu Asp Asp Leu Leu Glu Ile 185 gag gga ttc ggg aag ttt gat acg tct ttg gag aac att ctt aag ccg glu Gly phe Gly Lys phe Asp Thr ser Leu Glu Asn Ile Leu Lys pro 190 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg ccg aga ser ser ser Glu Thr Lys Asn Lys Tyr Pro Gln Tyr Arg Arg Pro Pro Arg 215 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1205 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1205 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 1205 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca 235 225 atg att cct gat atg att ctg cct 1255	Gly Gln Trp Ash val Leu Plo Ash Asp Ata 250 . 155	965
ttt gga ctg aat att ctt cgc atc gtt gda gdc gdc ctc ttg gga gda leu leu Glu Ile gag gga ttc ggg aag ttt gat acg tct ttg gag aac att ctt aag ccg Glu Gly Phe Gly Lys Phe Asp Thr 190 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg ccg aga 200 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg ccg aga 215 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca Met Ile Tyr Asp Leu Pro Asp Glu Lys Val Ser Phe Ser Phe Pro Ala 220 225 1109 1209	Glu Glu val Sel Ala Plo val Sel 1150	
gag gga ttc ggg aag ttt gat acg tct ttg gag adc att ctt day Pro Glu Gly Phe Gly Lys Phe Asp Thr ser Leu Glu Asn Ile Leu Lys Pro 190 agc tcc gag aca aaa aat aaa tat ccg caa tac cgc agg ccg ccg aga Ser Ser Glu Thr Lys Asn Lys Tyr Pro Gln Tyr Arg Arg Pro Pro Arg 205 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca Met Ile Tyr Asp Leu Pro Asp Glu Lys Val Ser Phe Ser Phe Pro Ala 220 1157	Phe Gly Leu Ash Tie Leu Arg Tie 180	
agc tcc gag aca aaa aat aaa tat ccg caa tac cg agg ccg agg ccg sos Arg Ser Ser Glu Thr Lys Asn Lys Tyr Pro Gln Tyr Arg Arg Pro Pro Arg 215 atg att tac gac ctg ccg gat gaa aaa gta tcc ttc agc ttc ccg gca Met Ile Tyr Asp Leu Pro Asp Glu Lys Val Ser Phe Ser Phe Pro Ala 235 220 221 222 223 225 226 227 228 229 220 220 221 222 223 225 226 227 228 229 220 220 220 220 221 220 220	Glu Gly Phe Gly Lys Phe Asp 105	
atg att tac gac ctg ccg gat gaa aaa gta tcc ctc age the Pro Ala Met Ile Tyr Asp Leu Pro Asp Glu Lys Val Ser Phe Ser Phe Pro Ala 235 220 225 230 235 226 227 228	Ser Ser Glu Thr Lys Ash Lys Tyl Flo Gli 17, 215	1157
and AAC CT3 TOO LIU ALU ALL CEM TO	Met Tie Tyr Asp Leu Pro Asp Gru 193 va. 230 235	1205
GÎN GIU SER ÂSP GIY ASP ASN ARG GIY LEU IPP LEU MEL ITE 250 250 240	cag gaa agc gac gga gac aac aga ggc cta tgg ctg atg att ctg cct Gln Glu Ser Asp Gly Asp Asn Arg Gly Leu Trp Leu Met Ile Leu Pro 240 245	1253

ccg Pro	ctc Leu	gtc Val	Met	ctg Leu	atc Ile	gtc Val	atg	Gly	atc	gtg	gcg	ctc	Ile	cag Gln	ccg Pro	130	1
cgg Arg	ggg Gly	Ile	255 ttt Phe	atc Ile	atc Ile	gtt Val	ser	260 ctt Leu	gcg Ala	atg Met	ttt Phe	atg Met	265 atg Met	acg Thr	ctg Leu	134	9
att Ile	Thr	tca Ser	acc Thr	gtg Val	cag Gln	Tyr	275 ttc Phe	cgc Arg	gac Asp	aaa Lys	Asn	Gln	cgt Arg	aaa Lys	aaa Lys	139	7
Arg	285 gaa Glu	gaa Glu	aaa Lys	aga Arg	G]u	290 cgg Arg	gtc Val	tat Tyr	acc Thr	Leu	Tyr	ctt	gaa Glu	aac Asn	Lys	144	5
300 aag Lys	aaa Lys	gag Glu	ctg Leu	cat His 320	gaa Glu	ctt Leu	gca Ala	gaa Glu	aga Arg 325	310 caa Gln	aag	ttc Phe	gta Val	ctt Leu 330	315 gat Asp	1493	3
ttc Phe	cat His	ttt Phe	cct Pro 335	aca	ttt Phe	gag Glu	aga Arg	atg Met 340	aaa	tat Tyr	tta Leu	aca Thr	aag Lys 345	qaq	atc Ile	1541	1
agc Ser	gga Gly	cga Arg 350	att Ile	tgg Trp	gaa Glu	aaa Lys	tcg ser 355	att Ile	gaa Glu	agc Ser	gcc Ala	gat Asp 360	ttt Phe	ctg Leu	caa Gln	1589	€
atc Ile	cgc Arg 365	ctt Leu	gga Gly	acg Thr	gga Gly	aat Asn 370	gtt Val	gca Ala	tct Ser	tcg Ser	tac Tyr 375	caa Gln	atc Ile	aat Asn	ttg Leu	1637	7
aac Asn 380	ggc Gly	gga Gly	gat Asp	ttg Leu	gcc Ala 385	aac Asn	cgc Arg	gat Asp	aca Thr	gac Asp 390	cat His	ctc Leu	ctt Leu	gaa Glu	caa Gln 395	1685	5
acg Thr	caa Gln	aaa Lys	atg Met	gaa Glu 400	gag Glu	gtc Val	tac Tyr	aga Arg	gag Glu 405	ctg Ŀeu	aaa ·Lys	aat Asn	gcg Ala	ccg Pro 410	atc ·Ile	1733	}
act Thr	gtg Val	aat Asn	ctt Leu 415	gcc Ala	gaa Glu	ggc Gly	ccg Pro	atg Met 420	ggc Gly	gtc Val	gtc Val	gga Gly	aaa Lys 425	ttg Leu	tcc Ser	1781	L
gtc Val	gtç Val	aaa Lys 430	aat Asn	gaa Glu	att Ile	cat His	cag Gln 435	ctt Leu	gtc Val	ggc	cag Gln	ctc Leu 440	gca Ala	ttt Phe	ttc Phe	1829)
cac His	agc Ser 445	tat Tyr	cat His	gac Asp	ttg Leu	cgc Arg 450	ttt Phe	gtc Val	ttt Phe	att Ile	ttt Phe 455	gac Asp	gaa Glu	gcc Ala	gag Glu	1877	,
tat Tyr 460	caa Gln	gaa Glu	tgg Trp	gaa Glu	tgg Trp 465	atg Met	aag Lys	tgg Trp	ctc Leu	ccg Pro 470	cat His	ttt Phe	cag Gln	atg Met	cct Pro 475	1925	I
cat His	att Ile	tat Tyr	gcg Ala	aaa Lys 480	ggg Gly	ttt Phe	att Ile	tac Tyr	aac Asn 485	gaa Glu	cag Gln	acg Thr	aga Arg	gat Asp 490	cag Gln	1973	
ctc Leu	ctt Leu	tca Ser	agc Ser 495	ata Ile	tat Tyr	gag Glu	att Ile	ttg Leu 500	aga Arg	gaa Glu	cgg Arg	gat Asp	tta Leu 505	gat Asp	gaa Glu	2021	
aac Asn	aaa Lys	aag Lys 510	aag Lys	act Thr	ttg Leu	ttt Phe	aag Lys 515	ccg Pro	cac His	ttt Phe	gtg Val	ttt Phe 520	atc Ile	atc Ile	aca Thr	2069	

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aat Asn	cag Gln 525	cag Gln	ctc Leu	atc Ile	gca Ala	gaa Glu 530	1112		tc :	1+	.ST2 tta Leu	gaa	ta	at t /r l	ctg _eu	gaa Glu	gg G1	c y	2117
aag Lys 540	cag Gln	aaa Lys	cac His	ctc Leu	gga Gly 545	gtg Val	tcg Sei	j a T	ca i	atc Ile	gtg Val 550	gcg Ala	gg A	ca g la d	gag Glu	aca Thr	aa Ly 55	a s 5	2165
gaa Glu	agc Ser	ctg Leu	tcc Ser	gaa Glu 560	ASI	att Ile	ca Hi	t a	111	ctt Leu 565	gtt Val	cgt Arg	ta T <u>y</u>	at a	att Ile	act Thr 570	ga Gl	a u	2213
cag Gln	gaa Glu	ggc Gly	gac Asp 575	TIE	ctg Leu	ato Ile	aa Ly	5 6	aa 1n 80	aag Lys	aaa Lys	gcc Ala	g V		cag Gln 585	atc Ile	cc Pr	.g .o	2261
ttt Phe	cag Gln	ctg Leu 590	ASP	cac His	cac His	aa Asi	ag n Ar 59	y v	gaa Slu	gac Asp	aac Asn	gaa Glu	. =	ag In 00	tťt Phe	tcc Ser	C <u>c</u> Ar	99 ' 9	2309
acg Thr	ctg Leu 605	aga Arg	acg Thr	ctt Lei	gac ı Asr	: са ні: 61	5 61	g a n T	acg Thr	ggc Gly	atg Met	acc Thr 615	_ ′ •	at sn	tcg Ser	att Ile	CC Pr	ro	2357
gat Asp 620	acc Thr	gta Val	tcg Sei	tti Pho	cto Lei 62!	וטנ	a ct u Le	g 1 u l	ttc Phe	caa Gln	gtg Val 630		g g s G	aa lu	gtc Val	gat Asp	ga A: 6:	ac sp 35	2405
ato Ile	ggc Gly	ato Ile	gaa e Gl	a caa u Gli 64	п цу	a tg s Tr	g at p Me	g :	aca Thr	agc ser 645	0.0	se	g g r A	gcc Ala	aaa Lys	tct Ser 650	_	tg eu	2453
gcc	gtç val	CC! Pro	g at 5 Il 65	e Gi	c ta y Ty	t aa r Ly	a gg s G	y	aaa Lys 660	gac Asp	gac Asp	at Il	t g e \	gtt /al	tat Tyr 665		a a ı A	ac sn	2501
Lei	cac His	67	и Ly О	SAI	а ні	5 G i	6	75		019			(680					2549
Gly	t tcg y Sei 68!	r G1	у Lу	s se	rGI	u Pi	90	Eu	Gin			69	5						2597
Va 70		s Ph	ент	S Pr	70	5	uv	a 1	714	7 110	71	ō - ·			•		7	15	2645
GI	g gg y Gl	y Gi	у ме	72	20	II F	0 1	110	~' 9	72	5 -	•				73	0		2693
Th	g at r Il	e Tr	7.	35 35	ie G	iu G	iy 3	C 1	740)					74	5			2741
ΑÌ	g to a Se	r I 75	ie Ly 50	ys 5	er G	iu L	7	55 55	Lys	, Ai	g a.		. 9	760)		•		2789
Ту	ic aa r Ly 76	rs Va 55	al A	sn H	15 I	7	70	ιsμ	, ,	,,,,	, <u>L</u> y	7	75	.,.	,	_		-	2837
ΓŽ	aa go /s Al	g aa la L	aa a ys T	cg g hr A	1a M	tg c et P 85	cg (ro H	ac Iis	ct1	t tt u Ph	c tt e Le 79		tt le	tca Sei	a ga r As	c ga p G	aa lu	rtt Phe 795	2885
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gcc Ala	gag Glu	ctg Leu	aaa Lys	agc Ser 800	gaa Glu	gaa Glu	cca	1029! gaa Glu	ttt	atc	cac	qaq	ctt Leu	gtc Val 810	agt Ser	2933
gcg Ala	gca Ala	agg Arg	atc Ile 815	ggg Gly	cga Arg	agc Ser	ctc Leu	ggg Gly 820	gtg Val	cac His	tta Leu	atc Ile	ttg Leu 825	gcg Ala	acg Thr	2981
caa Gln	aaa Lys	ccg Pro 830	ggc Gly	ggc Gly	atc Ile	atc Ile	gat Asp 835	gac Asp	cag Gln	att Ile	tgg Trp	agc ser 840	aac Asn	tcc Ser	aga Arg	3029
ttc Phe	aag Lys 845	gtc Val	gcc Ala	ttg Leu	aag Lys	gtg Val 850	cag Gln	gat Asp	gcg Ala	aat Asn	gac Asp 855	agt Ser	aaa Lys	gag Glu	atc Ile	3077
ctc Leu 860	aaa Lys	aac Asn	ggg Gly	gat Asp	gcg Ala 865	gct Ala	acc Thr	atc Ile	acg Thr	gta Val 870	acg Thr	ggc Gly	cgc Arg	ggc Gly	tat Tyr 875	3125
ttg Leu	caa Gln	gtc Val	ggc Gly	aac Asn 880	aac Asn	gag Glu	gtg Val	tat Tyr	gaa Glu 885	ctg Leu	ttc Phe	cag Gln	tct Ser	gca Ala 890	tgg Trp	3173
agc Ser	gga Gly	gcc Ala	cct Pro 895	tac Tyr	atg Met	gag Glu	gac Asp	ggc Gly 900	tac Tyr	ggc Gly	aca Thr	gag Glu	gat Asp 905	gaa Glu	gtg Val	3221
gcg Ala	atc Ile	gtc Val 910	aca Thr	gat Asp	acc Thr	gga Gly	tta Leu 915	att Ile	cct Pro	tta Leu	tca Ser	gat Asp 920	gtt Val	gat Asp	gct Ala	3269
gat Asp	cgc Arg 925	gct Ala	gcg Ala	aaa Lys	aaa Lys	gag Glu 930	gct Ala	gtg Val	acg Thr	gaa Glu	att Ile 935	tcg Ser	gca Ala	gtc Val	gtc Val	3317
gaa Glu 940	caa Gln	att Ile	gaa Glu	cgg Arg	att Ile 945	caa Gln	gcg Ala	gag Glu	atg Met	gga Gly 950	atc Ile	gag Glu	aag Lys	ctc Leu	ccg Pro 955	3365
agc Ser	cct Pro	tgg Trp	ctg Leu	ccg Pro 960	ccg Pro	ctt Leu	gaa Glu	gaa Glu	cgc Arg 965	ata Ile	ccg Pro	aaa Lys	acg Thr	cgc Arg 970	tat Tyr	3413
ccg Pro	tcg Ser	gag Glu	gaa Glu 975	gcc Ala	gat Asp	gcc Ala	ttt Phe	aac Asn 980	ttt Phe	gcc Ala	tat Tyr	atc Ile	gat Asp 985	Glu	cct Pro	3461
gaa Glu	aag Lys	caa Gln 990	agc Ser	cag Gln	gag Glu	ccg Pro	atc Ile 995	agc Ser	tac Tyr	cgc Arg	atg Met	atg Met 100	Ğ١	a ga u As	c ggc p Gly	3509
	atc Ile 100	_ G1;	c at y Il	c gt e Va	c ggo 1 Gly	5 tc 5 Se 10	r s	ca g er G	gc t ly T	ac g yr G	ly L	aa ys 015	tcc Ser	ctg Leu	aca Thr	3554
	acg Thr 1020	Th	g tt r Ph	c ate	g ate	g ag t se 10	r P	tt g he A	cc g la G	aa c lu G	ln Ţ	at yr 030	acg Thr	ccg Pro	gaa Glu	3599
	ttg Leu 103	Hi:	t ta s Ty	c ta r Ty	c at r Il	t tt e Ph 10	e A	ac t sp P	tt g he G	gc a ly A	sn G	ga 1y 045		ctg Leu		3644
	ctt Leu 105	Ā٦	a ag a Ar	g ct g Le	t ccg u Pro	g ca 5 Hi 10	s T	cc g hr A	cg g la A	at t sp T	yr P	tc he 060	ctg Leu	atg Met .	gac Asp	3689

caa	acq	aga	aaa	atc	gag	aaa	ttt	atq	204.s gt <u>c</u>	cgg	atc			gaa		3734
Gln	Thr 1065	Arg	Lys	Ile	Glu	Lys 1070	Phe	Met	vaı	Arg	1075	Lys				2770
atc Ile	gag Glu 1080	cac His	agg Arg	aaa Lys	aat Asn	ctc Leu 1085	ttc Phe	cgt Arg	gca Ala	aaa Lys	gaa Glu 1090	atc Ile	agc Ser			3779
_	aag Lys 1095	atg Met	tac Tyr	aat Asn	gcg Ala	ctg Leu 1100	aat Asn	gag Glu	gaa Glu	aag Lys	ctg Leu 1105	ccg Pro	ttt Phe	att Ile		3824
ttc Phe	ata Ile 1110	acg Thr	gtc Val	gac Asp	aac Asn	ttt Phe 1115	gac Asp	atc Ile	att Ile	aaa Lys	gac Asp 1120	gaa Glu	atg Met	cat His		3869
gaa Glu	ctc Leu 1125	gaa Glu	agc Ser	gaa Glu	ttt Phe	atc Ile 1130	cag Gln	ttt Phe	tca Ser	cga Arg	gac Asp 1135	ggc Gly	cag Gln	tcg Ser		3914
ctt Leu	gga Gly 1140	Ile	tat Tyr	tta Leu	atc	ctg Leu 1145	acc Thr	gcg Ala	aca Thr	agg Arg	gtc val 1150	aat Asn	gca Ala	atc Ile		3959
aga Arg	cag Gln 1155	Ser	ctc Leu	ttg Leu	aac Asn	aac Asn 1160	ctg Leu	aaa Lys	acg Thr	agg Arg	gtt Val 1165	gtc val	cac His	tat Tyr		4004
ctg Leu	atg Met 1170	Asp	cag Gln	tct Ser	gaa Glu	gca Ala 1175	tat Tyr	tcg Ser	att Ile	atc Ile	gga Gly 1180	agg Arg	ccg Pro	gaa Glu		4049
ttc Phe	agc Ser 1185	Leu	gaa Glu	ccg Pro	atc Ile	cct Pro 1190	gga Gly	cgc Arg	gtt Val	att Ile	atc Ile 1195	aat As n	aaa Lys	gaa Glu		4094
	caa Gln 1200	Tyr	ttc Phe	gca Ala	caa Gln	atg Met 1205	ttt Phe	atg Met	cct Pro	gtg Val	gaa · Glu 12 1 0	Ala	gac ·Asp	aac Asn	•	4139
gat Asp	atc Ile 1215	Glu	ctg Leu	ttt Phe	gaa Glu	ggg Gly 1220	Ile	aaa Lys	gcc Ala	gac Asp	att Ile 1225	GIII	gcg Ala	atc Ile		4184
gca Ala	gaa Glu 1230	Arg	tcg Ser	gaa Glu	ggc	atg Met 1235	Arg	aag Lys	ccg Pro	gcg Ala	cct Pro 1240	vai	ccg Pro	atg Met		4229
ctç Lei	ccg Pro 124	Let	gag Glu	ctt Leu	tcc Ser	gtc Val 1250	Thr	cag Gln	ttt Phe	gtg Val	aga Arg 1255	ASP	tat Tyr	ccg Pro		4274
	cag Gln 1260	Pro	gaa Glu	aga Arg	ggc Gly	ctt Leu 1265	Tie	cca Pro	atg Met	gga Gly	ctċ Leu 1270	wsh	gaa Glu	gaa Glu		4319
act Thi	gtc Val 127	Ğ٦١	a cço i Pro	gta Val	a tac I Tyr	ttt Phe 1280	Asn	ctt Leu	gag Glu	aaa Lys	aat Asn 1285	Lys	cac His	tgc Cys		4364
cto	att Ile 129	Met	g gg1 E Gly	caç / Gli	g acc	cag Gln 1295	Arg	gga Gly	aaa / Lys	aca Thr	aac Asn 1300	Vai	ato Ile	aag Lys		4409
ate Ile	c atg e Met 130	Lei	gaq u Gli	g cad u His	ctg s Lei	ctt Leu 1310	Asp	cat His	gac S Asp	ac <u>c</u> Thr	aaa Lys 131	_ Lys	ato : Ile	gcc Ala		4454

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Lys Gln Lys Thr Gln Arg Lys Pro Lys Val Ser Glu Glu Pro Val Gln 235 230 240

Glu Ala Asp Leu Asp Pro Asp Pro Val Ile Gln Ser Glu Pro Ile Ile 250 255

Ser Ser Phe Ser Asp Arg Asp Glu Lys Pro Glu Val Gln Ala Tyr Glu 260 265 270

Ala Pro Ala Ala Pro Ala Glu Pro Pro Ala Glu Pro Glu Ile Gly Glu 285 275

Glu Met Gln Ala Ser Gly Ala Pro Glu Ile Thr Phe Thr Glu Leu Glu 290 295

Asn Lys Asp Tyr Gln Leu Pro Ser Ile Gln Leu Leu Asp Asp Pro Lys 305 310 320

His Thr Gly Gln Gln Ala Asp Lys Lys Asn Ile Tyr Asp Asn Ala Arg 335

Lys Leu Glu Arg Thr Phe Gln Ser Phe Gly Val Lys Ala Lys Val Thr 340 345

Gln Val His Leu Gly Pro Ala Val Thr Lys Tyr Glu Val Tyr Pro Asp 365

Val Gly Val Lys Val Ser Lys Ile Val Asn Leu Ser Asp Asp Leu Ala 370 375

Leu Ala Leu Ala Ala Lys Asp Ile Arg Ile Glu Ala Pro Ile Pro Gly 385 390 395

Lys Ser Ala Ile Gly Ile Glu Val Pro Asn Ala Glu Val Ala Met Val 415 415

Ser Leu Lys Glu Val Leu Glu Ser Lys Leu Asn Asp Arg Pro Asp Ala 420 425

Lys Leu Met Ile Gly Leu Gly Arg Asn Ile Ser Gly Glu Ala Val Leu 435 440 445 Ala Glu Leu Asn Lys Met Pro His Leu Leu Val Ala Gly Ala Thr Gly 450 460 Ser Gly Lys Ser Val Cys Val Asn Gly Ile Ile Thr Ser Ile Leu Met 465 470 475 480 Arg Ala Lys Pro His Glu Val Lys Met Met Met Ile Asp Pro Lys Met 485 490 495 Val Glu Leu Asn Val Tyr Asn Gly Ile Pro His Leu Leu Ala Pro Val 500 505 510 Val Thr Asp Pro Lys Lys Ala Ser Gln Ala Leu Lys Lys Val Val Asn 515 520 525 Glu Met Glu Arg Arg Tyr Glu Leu Phe Ser His Thr Gly Thr Arg Asn 530 540 Ile Glu Gly Tyr Asn Asp Tyr Ile Lys Arg Met Asn Ala Ala Glu Glu 545 550 560 Ala Lys Gln Pro Glu Leu Pro Tyr Ile Ile Val Ile Val Asp Glu Leu 565 570 575 Ala Asp·Leu Met Met Val Ala Ser Ser Asp Val Glu Asp Ser Ile Thr 580 585 590 Arg Leu Ser Gln Met Ala Arg Ala Ala Gly Ile His Leu Ile Ile Ala 595 600 605 Thr Gln Arg Pro Ser Val Asp Val Ile Thr Gly Val Ile Lys Ala Asn 610 615 620 Ile Pro Ser Arg Ile Ala Phe Ser Val Ser Ser Gln Thr Asp Ser Arg 625 630 635 Thr Ile Leu Asp Met Gly Gly Ala Glu Lys Leu Leu Gly Arg Gly Asp 645 650 655 Met Leu Phe Leu Pro Val Gly Ala Asn Lys Pro Leu Arg Val Gln Gly
660 665 670 Ala Phe Leu Ser Asp Glu Glu Val Glu Lys Val Val Asp His Val Ile 675 680 685 Ser Gln Gln Lys Ala Gln Tyr Gln Glu Glu Met Ile Pro Glu Glu Thr

Gln Glu Thr Val Ser Glu Val Thr Asp Asp Leu Tyr Asp Glu Ala Val 705 710 720

Ala Leu Val Val Ser Met Gln Thr Ala Ser Val Ser Met Leu Gln Arg 735 730 735

Arg Phe Arg Ile Gly Tyr Thr Arg Ala Ala Arg Leu Ile Asp Ala Met 740

Glu Glu Arg Gly Ile Val Gly Pro Tyr Glu Gly Ser Lys Pro Arg Glu
760
765

Val Leu Leu Ser Lys Glu Gln Tyr Glu Glu Leu Ser Ser 770 775 780

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: BACILLUS LICHENIFORMIS MUTANT HOST CELL

(57) Abstract: A Bacillus licheniformis mutant host cell comprising a mutation (deletion) in one or more genes encoding polypeptides involved in sporulation wherein the mutant host cell expresses at least 5% less of the one or more polypeptides involved in sporulation than the parent host cell, when cultivated under comparable conditions. The mutant host cell is used for producing heterologous polypeptides.

PCT/DK 03/00200

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/75 C12N1/21 C07K14/32 According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12N C07K IPC 7 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to claim No. Citation of document, with indication, where appropriate, of the relevant passages Category * 1-21 WO 97 03185 A (NOVONORDISK AS ; PRIEST Х FERGUS G (GB); FLEMING ALASTAIR B (GB); TAN) 30 January 1997 (1997-01-30) the whole document 1-21 "Extracellular enzyme FLEMING A B ET AL: X synthesis in a sporulation-deficient strain of Bacillus licheniformis." APPLIED AND ENVIRONMENTAL MICROBIOLOGY vol. 61, no. 11, November 1995 (1995-11), pages 3775-3780, XP002902964 ISSN: 0099-2240 abstract 1-21 WO 98 22598 A (NOVO NORDISK BIOTECH INC) Х 28 May 1998 (1998-05-28) page 6, line 8 - line 11; claims 1,2,17 -/--Patent family members are listed in annex. Further documents are listed in the continuation of box C. tater document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "E" earlier document but published on or after the international "L" document which may throw doubts on priority daim(s) or which is clied to establish the publication date of another citation or other special reason (as specified) 'Y" document of particular relevance; the claimed invention concurrent of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "O" document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of mailing of the international search report Date of the actual completion of the international search 28. 10. 2003 15 July 2003 Authorized officer Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswljk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Ida Christensen Fax: (+31-70) 340-3016

Interi al Application No
PCT/DK 03/00200

	ation) DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 02 00907 A (JOERGENSEN STEEN TROELS; OLSEN CARSTEN (DK); NOVOZYMES AS (DK); AN) 3 January 2002 (2002-01-03) claims	
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

International application No. PCT/DK 03/00200

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see additional sheet
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-21 (partially)
Remark on Protest The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1 : claims 1-21 (partially).

A Bacillus licheniformis mutant host cell, which is mutated in at least one gene encoding a polypeptide involved in sporulation, which polypeptide is at least 80% identical to the polypeptide shown in SEQ ID No: 2.

Invention 2 : claims 1-21 (partially).

A Bacillus licheniformis mutant host cell, which is mutated in at least one gene encoding a polypeptide involved in sporulation, which polypeptide is at least 80% identical to the polypeptide shown in SEQ ID No: 4.

Invention 3: claims 1-21 (partially).

A Bacillus licheniformis mutant host cell, which is mutated in at least one gene encoding a polypeptide involved in sporulation, which polypeptide is at least 80% identical to the polypeptide shown in SEQ ID No: 6

Invention 4 : claims 1-21 (partially).

A Bacillus licheniformis mutant host cell, which is mutated in at least one gene encoding a polypeptide involved in sporulation, which polypeptide is at least 80% identical to the polypeptide shown in SEQ ID No:

etc.... etc....

Invention 96 :claims 1-21 (partially).

A Bacillus licheniformis mutant host cell, which is mutated in at least one gene encoding a polypeptide involved in sporulation, which polypeptide is at least 80% identical to the polypeptide shown in SEQ ID No: 191.

Information on patent family members

International Application No PCT/DK 03/00200

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